Reproducible extraction of high quality DNA from 1-16 blood samples
GeneMole® p.14

PerfeCta™ qPCR FastMixes: improve productivity and throughput without compromising qPCR performance
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editorial

New partners, new products, new ideas ...

It is hard to believe that Easter is over and the spring and summer lie before us. To help you get the most out of this most productive time of the year we are pleased to issue the latest VWRbioMarke magazine. This is an exciting issue as we welcome new partners for 2008 including Bertin, Biovest, Lonza, Mole and Quanta each offering state of the art products in their fields; Calbiochem introduce the InhibitorSelect™ Protein Kinase Library I, and Thermo Scientific offer their new nanolitre dispensing system; plus ideas, innovations and applications to save time, get results and improve yields.

Make sure you have a look through our promotional flyer, the VWRbioMarke shop, many of the featured ideas include products that are on special offer – now!

Very best regards
The VWRbioMarke Team

P.S you will also be able to find on the web with additional downloads and other information where available. To make sure that you get all the news about these and other new items as well as special offers and new catalogues register on our website to get the VWR e-newsletter.

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C.B.S. Scientific
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Merck Biosciences
Mole Genetics
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Operon
Pall Life Sciences
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All ClearPAGE gels are made with a composite-polymer, primarily cross-linked polyacrylamide plus a small amount of agarose. They are about 10-times stronger than ordinary acrylamide gels. The strength eliminates tearing when handling, cracking on drying, and sticking when blotting. Shrinking and swelling is reduced up to 20-fold. The constant shape produces a long PHYSICAL shelf life as well (unlike ANY other product on the market), permitting a full range of gels with years of shelf life. When a gel swells, not only is straightness compromised, but resolution falls as well. Normal polyacrylamide gels want to swell due to polymer relaxation. Conversely, when a gel shrinks in 20% methanol (typical concentration in blot buffers), the pore sizes shrink as well, making transfer efficiency lower than the ClearPAGE gels. Most importantly, with our novel polymerisation system, they also provide the best quality separations (resolution and gel straightness) you can get. (See figure 1).

The ClearPAGE SDS and DNA/Native cassette gels use a single running buffer system. Both gel systems have a neutral pH for long CHEMICAL shelf life (like NuPAGE™ and other gels). The running buffer contains Triethanolamine-Tricine. For SDS PAGE, the buffer includes SDS. For Native and DNA PAGE, no SDS is included. The SDS gels contain a Triethanolamine-chloride buffer with SDS and the DNA/Native gels Tris-acetate. They show no change in the separation for at least 2 years. For those who like the dual running buffer option for the NuPAGE gels, there is a “MES-like” running buffer available (ClearPAGE Low Range SDS Run Buffer - see comparison of figures 2 and 3), whose primary advantage is 40% faster run times for gels up to 12%, as compared to their higher percentage equivalents run with standard run buffer.

In addition, there are now also ClearPAGE Tris-Glycine-SDS gels. With stable, strong, and elastic gels, the results are more reliable than any other product on the market. For SDS PAGE, ClearPAGE gels come in nine neutral pH percentages, covering separations from 1 to 1000 kD. There are also four neutral pH gel percentages for DNA or Native PAGE. The TGS range presently includes 3 gel percentages: 4-20%, 8-16% and 12% with separations matched to the NOVEX gels. This combination of buffers and polymer makes the ClearPAGE line much simpler than for example the range from Invitrogen, and well as higher performing.

In addition, there are now also the ClearPAGE “Classics” Tris-Glycine-SDS gels. With stable, strong, and elastic gels, the results are more reliable than any other product on the market. For SDS PAGE, ClearPAGE gels come in nine neutral pH percentages, covering separations from 1 to 1000 kD. There are also four neutral pH gel percentages for DNA or Native PAGE. The TGS range presently includes 3 gel percentages: 4-20%, 8-16% and 12% with separations matched to the NOVEX gels. This combination of buffers and polymer makes the ClearPAGE line much simpler than for example the range from Invitrogen, and well as higher performing.
RNA interference has become a widely used research tool. Increasingly, clinicians feel the need to use this method, but often lack the expertise, equipment and personnel to run RNAi screens. RNAx introduces a short synopsis of how we helped our colleagues at the Charité to conduct a RNA interference screen for essential and drug sensitising genes in skin cancer cells.

Skin cancers (melanoma, epithelial skin tumours and cutaneous lymphomas) are among the major cancers affecting large parts of the populations with some of the highest increases in incidence rates. Most forms of epithelial skin tumours are not immediately life-threatening. However, about 100 per 100,000 individuals are diagnosed each year with malignant forms of these skin cancers. The therapeutic options for skin cancers are limited and palliative in most cases. In fact, whereas very early stages of melanoma and BCC can be surgically eliminated and some precancerous in situ (field) lesions of epithelial skin cancers cured by immune-modulating treatment, there is no curative therapy for manifest skin cancers of any of the three classes.

In order to gain a deeper understanding of skin cancers and to identify new therapeutic targets, for the group of Professor Peter Walden in the department of Dermatology, Venerology and Allergy at the Charité – University Medicine in Berlin, the platform has been used to perform an RNAi screen to identify essential as well as drug sensitising genes in melanoma cells.

To establish appropriate cellular models for the identification of new drug targets, three melanoma cell lines were analysed for their susceptibility to siRNA transfection. As shown in Figure 1, in all three cell lines the expression levels of the target mRNA could be suppressed by more than 97%, ensuring that RNAi based loss-of-function approaches are feasible in melanoma cells.

A promising approach to treat cancer is to combine several drugs exerting synergistic effects. For this purpose, cells have been treated with the chemotherapeutic drug Etoposide, leading to a nearly 50% reduction of cells in allstars, non-silencing control samples. In contrast to this, reduction of cell number was less prominent in samples where gene knockdown itself was already toxic.
Molecular biology

**RNAx**

In a pilot study to prepare for a genome wide screen, a library targeting 418 apoptosis related genes was screened. For this, two siRNAs targeting each gene were pooled into one well. The screening experiments were repeated three times independently. Cell numbers of non-treated and treated samples of each plate were normalised against the mean values of four allstars-treated samples. We could identify 40 genes reducing cell number by more than 65% and 9 genes, whose knockdown increases cell number by more than 15%. The statistical significance of the phenotypes were proven by student’s T-test.

In addition, knockdown of 15 genes exert significant reductions of cell numbers only after Etoposide induction, providing first hints for synergistic effects of combined drug treatments. In cases where Etoposide treatment had no effect on cell numbers at all, important factors for Etoposide-induced cell death might have been hit.

**Hit Validation**

It is well known that siRNAs can exert off-target effects. These are mainly due to three major reasons: (1) Activation of interferon responses, (2) Knockdown of mRNAs with sequence homologies to the intended target, and (3) Displacement of endogenous miRNAs from RISC by the transfected siRNA leading to the abolishment of miRNA functions.

In order to sort out primary hits related to off-target effects, we repeated the experiments with four additional siRNAs per putative target. As expected for a large number of the hits, primary phenotypes could not be confirmed. However for nine genes whose knockdown led to reduced cell numbers in the primary screen, with two or more out of four siRNAs, the phenotypes could be reproduced. Furthermore, for three genes, increased cell numbers after knockdown could be seen with at least two out of four siRNAs. Additionally, knockdown of six genes sensitised cells for Etoposide underscoring the reliability of the screening approach to identify targets for combinatorial cancer therapies.

For more information please contact your local VWRbioSciences specialist.
Bertin Technologies has designed a new generation homogeniser using bead-beating technology. This system, Precellys-24 (Figure 1 and Figure 2), allows homogenisation of a large range of biological samples from soft to hard and even elastic, simply by varying the bead type and speed. A figure of 8 motion rapidly gyrates the beads to grind from 1 to 24 samples in individual sealed tubes ensuring no cross contamination doubt between the tests. These tubes also make sample storage easy. Handling 24 samples in one sample preparation step means that processes can be very fast with 200 trials being homogenised in just 30 minutes.

Tissue homogenisation is a key process for Pharmacokinetic ADME and Pharmacology study tissue sample analysis using HPLC-tandem Mass Spectrometer system (LC-MS/MS). Homogenisation simplicity and efficiency; cross-contamination, analyte recovery and stability; are important factors that affect the data quality and high throughput (HPT) productivity. The more traditional method of tissue homogenisation involves a mechanical shearing force process through blending or grinding that produces heat, which may cause analyte instability. Some fragile tissues, such as rat or mouse lymph node and tumours, are also very difficult to handle with mechanic blenders. These tiny pieces must be manually ground, which is a really time consuming.

An ADME study, with rat brain, gonad, heart, kidney, lung and spleen samples were performed to evaluate the versatility of the Bertin beads beating technology. Including a pharmacological study of tumour and lymph node samples were also used to investigate the reproducibility and analyte recovery of the method.

Experimental Procedure

1. Sample Preparation
All tissue samples were weighed out to around 0.5 g directly into the Precellys 2 ml bead tube. To 1 part weight of tissue, 1 or 2 parts weight of buffer (de-ionised water) were added. The sample tubes were then placed on the Precellys-24 for homogenisation based on the following protocols (Table 1). In this experiment, only metallic beads were used. An aliquot of 100 mg homogenate of each sample was weighted out into a 96 well 1 ml cluster tube. Blank matrix homogenate was also weighed out for the preparation of calibration standards (STD), quality control samples (QC) and matrix blanks (BLK) in the sample plate. To each 100 mg homogenate, 400 µL acetonitrile with internal standard (ISTD) was added for analyte extraction. After centrifugation at 3000 rpm for 20 minutes, the supernatant was injected onto a HPLC/Tandem Mass Spectrometer (LC-MS/MS) for sample analysis.

2. HPLC and Mass Spectrometry Conditions
An Agilent 1100 series HPLC pump was connected with Applied Biosystems Mass Spectrometer API4000 for drug candidate and metabolite analysis. Waters Xterra MS C18 (5 µm, 2.1 x 50 mm) analytical column was used for the chromatography. A 1-minute linear gradient of acetonitrile / 10 mM ammonium acetate was applied for the analyte elution with flow rate of 700 µl min⁻¹. Positive ions were acquired in the multiple reaction monitoring mode (MRM) under APCI ion source. Nebuliser temperature was set at 400 °C.

Results and Discussion

Productivity: The efficiency of tissue homogenisation was significantly improved by 5-20 fold depending on the tissue type. In this experiment, only tumour samples were tested for the productivity. For each tumour sample, at
least 1 minute was spent when using manual grinding. But it only took 1 minute to homogenise 24 samples when Precellys-24 was used (Figure 3). There was a 5-minute break time between batches. So only 7 minutes was needed to complete 24-48 samples.

Recovery: To evaluate the analyte recovery, tumour samples were cut into two portions before homogenisation. Three samples from each time point were tested either using manual blending or beads beating. No significant difference was observed between the two methods (Table 2).

Reproducibility: To evaluate the reproducibility, each tumour sample was cut into three portions and prepared in different analytical batches. The process was reproducible within analytical runs (Table 3).

Versatility: In this study, all types of tissues were successfully homogenised and analysed using an LC-MS/MS system. To quantitatively determine the analyte concentration, a nine non-zero level calibration curve at the linear range of 1-5000 ng/ml was prepared for each analytical run monitored by three levels of quality control samples at low, medium and high concentration. To accept the analytical analysis, the measured concentration of the standard at each level must be within ±20% of the nominal concentration. Figure 4 summarises the accuracy of the analytical run for each type of tissues, which demonstrated an excellent accuracy for all tissue sample analyses.

Conclusion

Precellys-24 is a suitable and reliable system for a wide range of small animal tissue or small size of tissue homogenisation. Sample size should be adjusted to be less than 0.5 g for harder tissues, such as tumour, kidney and heart. Precellys-24 is the best homogeniser for handling very fragile tissues, like rat lymph node and tumour samples. It is also the best choice for other tissues weighing around 20-50 mg. When the protocol is set up and validated, sample preparation process remains the same with no bias in analysis.

For more information please contact your VWR Sales Specialist

Table 1. Tissue Homogenisation Protocols

<table>
<thead>
<tr>
<th>Tissue type</th>
<th>Brain</th>
<th>Gonad</th>
<th>Heart</th>
<th>Kidney</th>
<th>Lung</th>
<th>Spleen</th>
<th>Lymph node</th>
<th>Tumour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original weight (g)</td>
<td>1-1,5</td>
<td>1,5-3</td>
<td>0,7-2</td>
<td>2-2,6</td>
<td>1-1,5</td>
<td>0,5-1</td>
<td>0,02-0,2</td>
<td>0,2-0,5</td>
</tr>
<tr>
<td>Sample weight (g)</td>
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<td>~ 0,5</td>
<td>~ 0,5</td>
<td>~ 0,5</td>
<td>~ 0,5</td>
<td>~ 0,5</td>
<td>~ 0,5</td>
<td>~ 0,5</td>
</tr>
<tr>
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<td>1 cycle</td>
<td>1 cycle</td>
<td>2 cycles</td>
<td>2 cycles</td>
<td>1 cycle</td>
<td>1 cycle</td>
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<td>rpm 25s</td>
<td>6500</td>
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<td>6500</td>
<td>6500</td>
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<td>6500</td>
</tr>
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</table>

Table 2. Analyte Recovery Comparison of Rat Tumor Samples

<table>
<thead>
<tr>
<th>Tissue type</th>
<th>Precellys-24</th>
<th>Manual Blending</th>
<th>Difference (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration Determined (ng/mL)</td>
<td>Time-point 1</td>
<td>Time-point 2</td>
<td>Time-point 3</td>
</tr>
<tr>
<td>Tumour (mean of 3)</td>
<td>17567</td>
<td>22867</td>
<td>5530</td>
</tr>
<tr>
<td>Mean</td>
<td>17567</td>
<td>22867</td>
<td>5530</td>
</tr>
<tr>
<td>S.D.</td>
<td>464</td>
<td>1746</td>
<td>943</td>
</tr>
<tr>
<td>CV%</td>
<td>2,64</td>
<td>7,64</td>
<td>17,06</td>
</tr>
</tbody>
</table>

Table 3. Reproducibility of Rat Tumour Sample Analysis

<table>
<thead>
<tr>
<th>Analysis Batch No.</th>
<th>Time-point 1</th>
<th>Time-point 2</th>
<th>Time-point 3</th>
<th>Time-point 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Run 1</td>
<td>17400</td>
<td>24200</td>
<td>4390</td>
<td>560</td>
</tr>
<tr>
<td>Run 2</td>
<td>17100</td>
<td>24000</td>
<td>5500</td>
<td>496</td>
</tr>
<tr>
<td>Run 3</td>
<td>18200</td>
<td>20400</td>
<td>6700</td>
<td>620</td>
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<tr>
<td>Mean</td>
<td>17567</td>
<td>22867</td>
<td>5530</td>
<td>559</td>
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<tr>
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<td>1746</td>
<td>943</td>
<td>50,6</td>
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<tr>
<td>CV%</td>
<td>2,64</td>
<td>7,64</td>
<td>17,06</td>
<td>9,06</td>
</tr>
</tbody>
</table>

For more information on these products contact your local VWR sales office, send an e-mail to vwbioimark@eu.vwr.com or visit our website www.vwr.com
BRAND ultra-thin 8-strip tubes and caps for PCR

Optimised protection against evaporation

PCR is the key technique in molecular genetics that permits the analysis of any short sequence of DNA (or RNA). PCR is a very efficient method used to amplify selected sections of DNA leading to a very high number of copies of DNA strands.

Among other things the efficiency of this application depends on

• Quality of the used raw material of disposables to avoid interactions with the reaction
• Thickness and uniformity of the walls for optimal transparency and consistent heat transfer
• Matching design between tubes and caps to reduce evaporation loss and minimise opening and closing forces

The new line of PCR Strips from BRAND, manufactured under most modern clean room conditions, offer a unique combination of new features!

New features:

• Reduced evaporation rates
• Uniform wall thickness for constant heat transfer and short cycles times
• Retaining pad at one side for easier handling of the 8-strip tubes
• Choose between domed caps or flat caps with highly transparent area for quantitative Real Time PCR
• Flange at the front of every cap for gentle opening - even when wearing gloves - to reduce the risk of contamination
• Made from 100% virgin, medical grade polypropylene (PP)
• Coloured tubes and caps for simple sample identification
• Certified to be free of RNase, DNase and DNA
For more information on these products contact your local VWR sales office, send an e-mail to vwrbiomarket@eu.vwr.com or visit our website www.vwr.com

Molecular biology

See our offer in VWRbioMarkShop!

Comparison of evaporation rates %

<table>
<thead>
<tr>
<th></th>
<th>flat caps</th>
<th>domed caps</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRAND</td>
<td>1,3</td>
<td>1,5</td>
</tr>
<tr>
<td>competitor</td>
<td>1,8</td>
<td>2,3</td>
</tr>
</tbody>
</table>

Strips of 8 PCR tubes and detached cap strips, autoclavable at 121 °C (20 min). PP. 8 connected 0,2 ml tubes. Cap strips (available separately) consisting of 8 domed or flat caps provide tight seal during all PCR cycles and protect samples from cross-contamination. They close and open easily without tools.

Pack of 125 strips, 1000 tubes or caps total.

<table>
<thead>
<tr>
<th>Capacity (ml)</th>
<th>Colour</th>
<th>Strips of 8 PCR tubes</th>
<th>Strips of 8 PCR caps, domed</th>
<th>Strips of 8 PCR caps, flat*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0,2</td>
<td>clear</td>
<td>211-3263</td>
<td>211-3265</td>
<td>731-1250</td>
</tr>
<tr>
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<td>green</td>
<td>211-3272</td>
<td>211-3276</td>
<td></td>
</tr>
<tr>
<td>0,2</td>
<td>blue</td>
<td>211-3273</td>
<td>211-3277</td>
<td></td>
</tr>
</tbody>
</table>

* flat caps suitable for Real Time PCR.
A new method to simultaneously isolate total RNA, DNA and protein

TY, Zhu, WK, Wu, Guo, Qi

Abstract

A new method is described for the simultaneous extraction of proteins and nucleic acids from the same experimental sample allowing for direct correlation between genetic and proteomic data. This procedure, using an innovative buffer system, requires no hazardous chemicals and provides a simple and effective way to analyse protein and nucleic acids simultaneously from a single biological material sample without affecting yield and quality. Using this method, protein can be isolated in as little as 20 minutes, RNA in as little as 30 minutes, and DNA as little as 40 minutes.

Introduction

For many clinical and research applications, fully understanding normal biological process such as cell differentiation, development and aging, and pathological conditions requires integrated genomic, transcriptional, and proteomic studies. It is critical to have the ability to isolate the RNA, DNA and proteins from the same biological sample.

A quick and reliable method to simultaneously extract DNA, RNA, and proteins from a single sample is ideal for the generation of matched samples saving time and money. It is especially important when the sample is very limited, for example in biopsies, and cannot be divided and used in separate isolation techniques for each component. A procedure for simultaneously isolating DNA, RNA and protein from the same biological sample is described in Coombs, L. M., et al. (1990). This method is disadvantageous because it is extremely time consuming (2-3 days for completion) and it requires an ultracentrifuge, which is an expensive piece of equipment that can process only a limited number and size of samples simultaneously. Another common procedure for simultaneously isolating DNA, RNA and Protein from the same biological sample is described in Chomczynski, (1993), it involves phenol–chloroform separation. Researchers are increasingly turning away from those classic RNA and protein extraction techniques because of the hazardous chemicals used, making the methods generally unsuitable for routine use in the lab environment.

Here, we report an efficient and reliable method for the simultaneous isolation of RNA, DNA and proteins from the same biological sample by using an innovative solution based buffer system. This method is less hazardous (does not involve phenol and chaotropic salts), and fast. Proteins, RNA and Genomic DNA can be isolated in less than 40 minutes.

Materials and Method

The E.Z.N.A.® SQ RNA/DNA/Protein Extraction Kit is supplied by Omega Biotek Inc. All experimental samples are provided by Zhongshan University. DNA, RNA and proteins were extracted using E.Z.N.A.® SQ RNA/DNA/Protein Kit by following the manufacturer’s instructions. Figure 1 shows the diagram of the protocol. Nine tissue samples used are described as following: 15 mg of Fish Liver, Frog Liver, Rat Liver, Fish Spleen, Rat Kidney, 5 x 106 Hela Cells, 100 mg fresh Tomato Leaves, 3 ml log phase bacterial cell (DH5a). After the purification, the DNA and RNA were analysed by running on 1,0% agarose gel and spectrophotometer. The proteins were analysed by running on SDS-PAGE Gel. The quality of DNA and RNA were further analysed by PCR or RT-PCR.

RT-PCR Analysis of RNA

2 µg of Total RNA was digested with RNase-free DNase and reverse transcribed with Random Primer. The optimal reverse transcription (RT) was carried out in 20 µl volumes consisting of 4 µl 5 x Reaction Buffer, 0,2 µg Random Primer, 2 µl 10 mM dNTP Mixture, 20 U Ribonuclease Inhibitor, 200 units of M-MuLV (MBI Foerments). Add 80 µl ddH2O to dilute cDNA and 1 µl of cDNA was used as template DNA. The PCR amplifications were performed in a volume of 50 µl: 1µl Taq Polymerase, 5 µl 10 x PCR Buffer, 0,5 mg/ml BSA, 0,25 mM dNTPs, 100 ng genomic DNA and 500 nM Primers. Amplification was carried out using the following Program: 3 min at 95 ºC; followed by 35 cycles of 30 s at 95 ºC, 30 s at 58 ºC, 1 min at 72 ºC, and a final extension of 10 min at 72 ºC.

Purification of Proteins, RNA and Genomic DNA: The samples were homogenised using a tissue homogeniser and lysed by Buffer SQ1. Purification of Proteins, RNA and Genomic DNA: The samples were homogenised using a tissue homogeniser and lysed by Buffer SQ1. Proteins were precipitated by the addition of Buffer SQ2. After the centrifugation to collect protein pellet, the supernatant was collected and isopropanol was added to precipitate RNA. After the centrifugation to collect RNA pellet, the supernatant was mixed with Buffer SQ3 to precipitate DNA. The RNA, DNA and protein pellets were dissolved with water.

Table 1. Spectrophotometer Analysis

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Fish Liver</td>
<td>1.89</td>
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<tr>
<td>Frog Liver</td>
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<tr>
<td>Hela Cells</td>
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<td>2.5</td>
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<td>Tomato Leaves</td>
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<tr>
<td>E.coli</td>
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<tr>
<td>DNA Sample</td>
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<tr>
<td>Fish Liver</td>
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<td>60</td>
</tr>
<tr>
<td>Frog Liver</td>
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<td>Fish Spleen</td>
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<tr>
<td>E.coli</td>
<td>2.01</td>
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<td>13</td>
</tr>
</tbody>
</table>
by 35 cycles of 30 s at 94 °C, 30 s at 55 °C, and 40 s at 72 °C and finally 10 min at 72 °C for the final extraction.

PCR Analysis of DNA

The genomic DNA was used directly for PCR. The PCR amplifications were performed in a volume of 50 μl: 1 μl Taq Polymerase, 5 μl 10 x PCR Buffer, 0.5 mg/ml BSA, 0.25 mM dNTPs, 100 ng genomic DNA and 500 nM Primers. Amplification was carried out using the following Program: 3 min at 95 °C; followed by 35 cycles of 30 s at 94 °C, 30 s at 55 °C, and 40 s at 72 °C and finally 10 min at 72 °C for the final extraction.

SDS-PAGE Analysis of Protein

300 μl 1x SDS PAGE Loading Buffer (40 mM Tris/HCl pH 6.8; 5% Mercaptoethanol; 1% SDS; 5% Glycerin; Bromophenol Blue.) were added into the Protein pellet and vortexed to dissolve the protein. The samples were boiled for 5 min in H₂O bath and then spin at 3,000 x g for 5 min. Transfer 12 μl of the supernatant into 10% SDS-PAGE Gel. After electrophoresis, the gel was stained by G250 Coomassie dye.

Results

Nucleic Acids Quantification and Quality were evaluated using a spectrophotometer (DU-640), and the 260:280 ratios were calculated. Nucleic acids integrity was analysed using agarose gel electrophoresis (Figure 2 and Figure 3). RNA ratios were shown to be between 1.8 and 2.1, while DNA ratios were between 1.6 and 1.8. Both DNA and RNA extracted from this method showed high quality (Table 1).

RT-PCR amplification

The quality of Purified RNA was further analysed by RT-PCR. Figure 4 shows that all RNA sample can successfully amplified by RT-PCR.

PCR Amplification

The genomic DNA isolated with this method was used directly for PCR. Figure 5 shows that all DNA can samples be successfully amplified by PCR.

SDS-PAGE Analysis

The profile of the protein was analysed by sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) followed by G250 Coomassie staining. As observed in Figure 6, proteins extracted with the E.Z.N.A.™ SQ RNA/DNA/Protein Kit yields a wide spectrum of proteins. Further test also confirmed the proteins purified with this method are suitable for Western Blot analysis (Data not shown).

Conclusion

E.Z.N.A.™ SQ RNA/DNA/Protein Extraction Kit provides a simple, safe, flexible, and effective way to simultaneously extract nucleic acids and proteins from same sample.


Preps (g) Cat. No.
1 OMEGR8042-00
4.5 OMEGR8042-01
18 OMEGR8042-02
37.5 OMEGR8042-03
75 OMEGR8042-04
Precast agarose solutions for RNA analysis

Rapid RNA Analysis with the FlashGel® System for RNA

High quality, intact RNA is essential for consistent results in gene expression techniques, Northern analysis, cDNA library construction, and cDNA labelling for microarrays. Most protocols recommend checking RNA integrity prior to downstream analysis using a denaturing agarose gel stained with ethidium bromide. Common problems with this technique are the risks of nuclease contamination and operator exposure to hazardous materials during gel preparation, and time required for gel preparation, electrophoresis, and staining. Frequently, RNA preparations do not provide sufficient yields to spare the amount required for detection on ethidium bromide gels.

RNA chip systems offer a faster, more sensitive alternative to gel analysis. However, these systems are costly and require significant protocol validation. The new FlashGel® System for RNA offers the speed, sensitivity and convenience of a chip, yet fits neatly into existing experimental flow, at a cost accessible to most research labs.

The FlashGel® System revolutionised DNA electrophoresis by offering 5 minute separation and real-time visualisation of sample migration. The FlashGel® System for RNA is optimised for the unique requirements of RNA samples. RNA analysis is complete in less than 30 minutes and requires one-fifth the amount of total RNA for detection. The FlashGel® System is recommended for verification and analysis of total RNA (Figure 1), quick checks of native RNA, and checks for RNA degradation.

Rapid RNA analysis

The FlashGel® System for RNA separates up to 34 samples of RNA in 8 minutes or less. RNA samples are visible on the FlashGel® Dock for up to 4 minutes, after which they fade and then reappear with increasing intensity following a 10-20 minute post-run hold. Full analysis is complete in less than 30 minutes, compared to the 1-3 hours required for typical agarose gels.

Sensitive detection

RNA quantities <10 ng per band are clearly detected on the FlashGel® System for RNA. Ethidium bromide gels require 200 ng of RNA for detection. Some RNA preparations deliver such low yields that there may not be the surplus RNA needed to assess integrity. High sensitivity stains such as GelStar® Stain (Lonza) and SYBR® Green II Stain (Lonza) offer detection sensitivity down to 2 ng per band. The FlashGel® System for RNA uses a similar stain that reduces the amount of required RNA by a factor of five or greater compared to...
For more information on these products contact your local VWR sales office, send an e-mail to vwbio@vwr.com or visit our website www.vwr.com

### Molecular biology

<table>
<thead>
<tr>
<th>FlashGel® RNA Cassette</th>
<th>Reliant® MOPS Gel</th>
<th>Hand cast agarose gel</th>
</tr>
</thead>
<tbody>
<tr>
<td>28 minutes, 8 min run with 20 minute hold</td>
<td>120 minutes, 60 min run with 60 min GelStar® Stain post-stain</td>
<td>60 minutes, 60 min run with EtBr in gel</td>
</tr>
</tbody>
</table>

**Figure 3.** Performance comparison of denatured RNA samples on agarose gels.

ethidium bromide. Figure 3 illustrates detection sensitivity of the FlashGel® System for RNA compared to other agarose gel methods.

**Clean, enclosed system**

The FlashGel® System for RNA eliminates the hazards and tedium associated with RNA gel preparation. The cassettes fully enclose the gel and running buffer, eliminating operator exposure to hazardous reagents, and protecting samples from contaminating RNases. The cassettes contain a 1.2% agarose and buffer blend which are selected for purity, manufactured in a dedicated clean room, and guaranteed RNase-free. The FlashGel® Dock provides electrophoresis and visualisation for both DNA and RNA Cassettes. The Dock does not come in contact with samples or gels, so it is not necessary to dedicate a unit for RNA work.

**Northern blotting or RNA recovery needed?**

For Northern blotting or RNA recovery, check out Reliant® or Latitude® RNA Precast gels. Reliant® and Latitude® Precast RNA Agarose Gels are precision cast in 1.25% SeaKem® Gold Agarose with MOPS buffer and are guaranteed RNase free.

### FlashGel® System for RNA Starter Pack

<table>
<thead>
<tr>
<th>Description</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contains: 9 cassettes 1.2%, 12 + 1 well, Sample Buffer, RNA Marker and Molecular Biology Water. Dock sold separately.</td>
<td>733-1612</td>
</tr>
<tr>
<td>FlashGel® Dock</td>
<td>733-1609</td>
</tr>
<tr>
<td>FlashGel® RNA Cassettes – 9/pk, 1.2% agarose, 12+1 single-tier</td>
<td>733-1617</td>
</tr>
<tr>
<td>FlashGel® RNA Cassettes – 9/pk, 1.2% agarose, 16+1 double-tier</td>
<td>733-1618</td>
</tr>
<tr>
<td>Formaldehyde Sample Buffer (contains bromophenol blue and xylene cyanol), supplied in 5 x 1 ml</td>
<td>733-1739</td>
</tr>
<tr>
<td>FlashGel® Loading Dye (contains xylene cyanol), supplied in 5 x 1 ml, 5X concentrate</td>
<td>733-1619</td>
</tr>
<tr>
<td>FlashGel® RNA Marker (0.5 Kb – 9 Kb), 50 μg (1 μg/ml)</td>
<td>733-1623</td>
</tr>
<tr>
<td>AccuGENE® Molecular Biology Water 1 L RNase Free</td>
<td>733-1631</td>
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### FlashGel® System for RNA Analysis Kits

<table>
<thead>
<tr>
<th>Description</th>
<th>No. of Wells</th>
<th>Buffer</th>
<th>Cat. No.</th>
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<tbody>
<tr>
<td>contains: gels and sample buffer described, RNA marker, 1 L 10X MOPS buffer, and 2 x 250 μl GelStar® Stain.</td>
<td>8 well</td>
<td>Formaldehyde sample buffer</td>
<td>733-1586</td>
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<tr>
<td>Reliant® RNA gel</td>
<td>20 well</td>
<td>Glyoxal sample buffer</td>
<td>733-1589</td>
</tr>
<tr>
<td>Latitude® RNA gel</td>
<td>8 well</td>
<td>Formaldehyde sample buffer</td>
<td>733-1587</td>
</tr>
<tr>
<td>20 well</td>
<td>Glyoxal sample buffer</td>
<td>733-1588</td>
<td></td>
</tr>
<tr>
<td>1,25% SeaKem® Gold</td>
<td>1 × 20 well</td>
<td>MOPS</td>
<td>733-1603</td>
</tr>
<tr>
<td>1,25% SeaKem® Gold</td>
<td>2 × 20 well</td>
<td>MOPS</td>
<td>733-1604</td>
</tr>
<tr>
<td>Latitude® RNA Analysis Kits</td>
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<tr>
<td>contains: gels and sample buffer described, RNA Marker, 1 L 10X MOPS buffer, and 2 x 250 μl GelStar® Stain.</td>
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<tr>
<td>1 x 20 well</td>
<td>Glyoxal sample buffer</td>
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</tr>
<tr>
<td>2 x 20 well</td>
<td>Glyoxal sample buffer</td>
<td>733-1608</td>
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</tbody>
</table>

**Sorry- Lonza products are not available in Finland or Norway**
GeneMole® automated system:
Reproducible extraction of high quality DNA

GeneMole® is a new bench-top automated system for extraction of nucleic acids. The system processes from 1 to 16 samples run and handles sample volumes ≤ 200 µl. Reagents are supplied in sealed strips (MoleStrips™) according to the “one-strip one-sample” principle.

Extraction pure DNA from whole blood is challenging. Blood from vertebrates, except birds, has few DNA containing cells (as little as 1-2%). The large amounts of haemoglobin and plasma proteins can clog up extraction matrixes and inhibit downstream applications. Plus there is always a risk that blood samples contain harmful infectious agents.

GeneMole® is a small scale automated system particularly suitable for extracting DNA from whole blood from any vertebrate. The gentle magnetic bead based separation technology extracts DNA suitable for most downstream applications. Problems like fragmentation of DNA and clogging, which are frequently encountered with spin columns, are avoided. The closed GeneMole® system also reduces exposure to potentially infected blood compared to any manual extraction method.

This report aims to demonstrate GeneMoles® ability to extract high quality DNA from blood from many different sources.

Methods
The GeneMole® instrument and MoleStrips™ DNA Blood (pre-filled reagent strips) were used to isolate DNA from blood drawn from humans and various domestic animals. DNA was extracted from 100 µl blood and then eluted in 200 µl Tris-HCl. Avian blood has nucleated erythrocytes which gives a very large amount of DNA per sample volume. The chicken samples were therefore diluted in PBS prior to extraction (4 µl blood diluted to a final volume of 200 µl). Fresh and frozen blood and blood samples stored in different anti-coagulants were tested. Aliquots of pure DNA were used as template for DNA sequencing, pyrosequencing, long-range PCR and quantitative real-time PCR.
**Results**

The yield and quality of the extracted DNA (measured by NanoDrop or standard spectrophotometer) are listed in table 1. High quality DNA was obtained from all the tested samples.

Automated DNA extraction gave highly reproducible results without any visible cross-contamination (figure 2). The DNA was free from inhibitors and compatible with downstream analysis methods like DNA sequencing (MegaBACE and ABI), multiplexed-, long-range- and quantitative real-time PCR (ABI). The DNA quality was also adequate for SNP typing by pyrosequencing (figure 3). For data on other downstream methods contact your local VWR specialist.

**Conclusion**

GeneMole® yields high quality DNA from human and domestic animal blood. Automation eliminates operator-to-operator variations, and the system provides some protection from infected blood samples. GeneMole® is therefore an ideal extraction system for laboratories with need for processing 1-32 samples per day, and it is suitable for genetic testing of humans and animals, veterinary diagnostics, biobanking and genetic research.

Data for domestic animals were kindly provided by Ulla Gustafson and Marie Wibe at Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences.

**NB!** GeneMole™ is not certified for IVD, and should only be used for research applications.

---

**Table 1.** Yield and quality of genomic DNA extracted from blood from different species by GeneMole®. The values are averages of 16 samples run in parallel (one donor). Yield depends on white blood cell counts, and will therefore vary from individual to individual.
PerfeCta™ qPCR FastMixes

Improve productivity and throughput without compromising qPCR performance

Fast-ramp rate qPCR instruments for 384 or more wells offer higher assay throughput through reduced cycling times as well as lower costs through reduced reaction volumes. Specific reagents have been designed for such fast cycling instruments to avoid compromising amplification efficiency, dynamic range or sensitivity. PerfeCta qPCR FastMixes have been specifically developed to address these needs for either SYBR® Green or probe-based detection chemistries.

PerfeCta qPCR FastMixes are 2X concentrated, ready-to-use reaction cocktails that deliver maximum PCR efficiency, sensitivity, specificity and robust fluorescent signal. FastMixes can be used with fast cycling protocols on either rapid ramp rate or conventional block real-time cyclers with your current primers and probes. Shorter reaction times get you your results quicker and maximise utilisation of shared qPCR instrument resources (Figure 1). PerfeCta qPCR FastMixes are available for all real-time PCR instrument platforms including those requiring normalisation with ROX reference dye.

An important component of all the PerfeCta FastMixes is AccuFast™ Taq DNA polymerase. This hot-start thermostable polymerase was developed to allow instantaneous activation at 95 ºC. It contains a proprietary mixture of monoclonal antibodies that bind to the polymerase and keep it inactive prior to the initial PCR denaturation step. When heated to 95 ºC, these antibodies are irreversibly inactivated during the initial PCR denaturation step. This rapid-release automatic hot-start helps maximise specificity in qPCR. Highly specific amplification is crucial to successful qPCR, particularly when using SYBR Green I dye technology as this dye binds to and detects any dsDNA generated during amplification. Rapid recovery of fully active, unmodified Taq DNA polymerase is critical for efficient extension kinetics. Replication of fragments up to 200 bp is complete in less than 20 s at 60 ºC. It is important to note that while chain extension may actually be completed in much less than 20 s, the minimum data collection time varies for different real-time PCR systems. This dwell time must be taken into consideration when choosing a particular cycling protocol to allow accurate collection and analysis of the fluorescent signal.

The physical properties of the intended DNA target are another factor that must be considered in designing a fast cycle program.

SYBR Green FastMixes

PerfeCta SYBR Green FastMixes, are provided as 2X concentrated, ready-to-use reaction cocktails that contains all components, except primers and template for real-time quantitative PCR on a variety of real-time PCR systems (See Table 2). This unique combination of proprietary buffer, stabilisers, and AccuFast™ Taq DNA polymerase delivers maximum PCR efficiency, sensitivity, specificity and robust fluorescent signal using fast, or conventional, cycling protocols with SYBR Green qPCR.

The performance of PerfeCta SYBR Green FastMix is identical to that for PerfeCta SYBR Green SuperMix, which provides best in class performance for conventional cycling protocols. Figure 2 shows a comparison of sensitivity and signal amplitude for detection of the ADAR mRNA in log-fold serial dilutions of cDNA from HeLa cell total RNA. This demonstrates that one can take advantage of faster cycling protocols and reduced reaction volumes on conventional real-time PCR systems without compromising sensitivity or detection or dynamic range.

**Table 1. qPCR Cycling Comparison**

<table>
<thead>
<tr>
<th>PCR Cycling Conditions</th>
<th>Conventional Reagents</th>
<th>PerfeCta™ FastMix™</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzyme Activation</td>
<td>2 to 15 min 95 ºC</td>
<td>20s, 95 ºC</td>
</tr>
<tr>
<td>PCR Cycling</td>
<td>15s, 95 ºC @60s, 60 ºC</td>
<td>1 – 3s, 95 ºC @60s, 60 ºC</td>
</tr>
<tr>
<td>Cycling Time</td>
<td>40 cycles = 52 min (excluding ramp time)</td>
<td>40 cycles = 14 min (excluding ramp time)</td>
</tr>
<tr>
<td>Standard Block Cycler</td>
<td>iCycler iQ®</td>
<td>Mastercycler® ep realplex</td>
</tr>
<tr>
<td>Fast Block Cycler</td>
<td>1 hr, 20 min to 1 hr, 43 min</td>
<td>1 hr, 12 min to 1 hr, 22 min</td>
</tr>
</tbody>
</table>

Do you want to try PerfeCta™ qPCR FastMixes? Contact your local VWR office, and they will provide you with samples.
For more information on these products contact your local VWR sales office, send an e-mail to verbiomarke@eu.vwr.com or visit our website www.vwr.com

Performance on a fast-ramp qPCR system (Eppendorf
Molecular biology

Figure 2. Fast-cycle SYBR® Green qPCR with equal performance to conventional SYBR Green protocols RNA-specific adenosine deaminase (ADAR) was amplified from log-fold dilutions of total HeLa cell cDNA (100 ng to 10 pg) using PerfeCta™ SYBR Green FastMix™, with fast cycling protocol, or PerfeCta™ SYBR Green SuperMix, with conventional cycling conditions on a Bio-Rad iCycler iQ.

Figure 3. Faster, More Sensitive SYBR Green qPCR GAPD was amplified from log-fold serial dilutions of qScript™ synthesised cDNA from HeLa cell total RNA (100 ng to 100 fg) with either PerfeCta™ SYBR® Green FastMix™ or a fast SYBR® Green PCR kit from Supplier Q using recommended conditions for each kit on an Eppendorf Mastercycler ep realplex equipped with a rapid ramp rate cycling block.

Figure 4. High-Performance TaqMan Results in Less Time and Lower Reaction Volumes. Large ribosomal protein (RPLPO) was amplified from log-fold serial dilutions of qScript™-synthesised cDNA from HeLa cell total RNA (100 ng to 100 fg) with either PerfeCta qPCR FastMix or an antibody-based hot-start Taq master mix from Supplier B according to each manufacturer’s recommended protocol using the RPLPO TaqMan Endogenous Control primer/probe set (Applied Biosystems). Average plots for quadruplicate reactions of each input quantity are shown. Ct standard curve plots and linear regression statistics are shown in the inset.

Components

- Primers
- Probes
- Template DNA
- Buffers
- enzymes
- Reagents
- Controls

Performance on a fast-ramp qPCR system (Eppendorf

Kit

qPCR FastMix low ROX
250RXN 733-1401
500RXN 733-1403
1250RXN 733-1402
250RXN 733-1397
500RXN 733-1399
1250RXN 733-1398
250RXN 733-1393
500RXN 733-1395
1250RXN 733-1394

SYBR Green FastMix low ROX
250RXN 733-1389
500RXN 733-1391
1250RXN 733-1390
250RXN 733-1385
500RXN 733-1387
1250RXN 733-1386
250RXN 733-1381
500RXN 733-1383
1250RXN 733-1382
250RXN 733-1377
500RXN 733-1379
1250RXN 733-1378

qPCR FastMixes for probe-based detection
PerfeCta qPCR FastMixes are 2X concentrated, ready-to-use reaction cocktail that contains all components, except primers, probe(s), and template for real-time quantitative. An added benefit offered by qPCR FastMixes is the inclusion of dUTP and uracil-N-glycosylase (UNG) to prevent amplification of carry-over contamination from previous dU-containing PCRs. During the development of this product we found superior fast-cycling performance with dUTP-containing formulations, particularly for MGB-modified TaqMan probes, as compared to equivalent dTTP mixes. The proprietary buffer, stabilisers, and AccuFast Taq DNA polymerase have been specifically optimised to deliver maximum PCR efficiency, sensitivity, and robust fluorescent signal with TaqMan® or TaqMan MGB probe chemistry when using rapid PCR cycle times and reduced reaction volumes. This affords greater reagent economy and laboratory throughput on conventional or rapid ramp rate qPCR systems. The enhanced specificity of this FastMix suppresses cross-reactivity between homologous sequences, improving detection and discrimination in SNP applications.
The Bioprocess Research lab at Oxford Brookes University is primarily concerned with new high-value bioproducts, and better ways of over-producing them. Currently we are focusing on the biosynthesis of surfactin, a lipo-heptapeptide with interesting surfactant properties. Although the majority of our work has been funded by the UK Engineering & Physical Sciences Research Council to develop foam fractionation as a physical method for separating biosurfactant molecules from culture broths, we have become increasingly interested in the way in which surfactin is regulated at the molecular level.

Surfactin is produced by bacteria in the genus Bacillus, most notably *Bacillus subtilis*. Each of the seven amino acids that make up surfactin are joined together by large cytoplasmic enzymes, with no DNA template. This process of non-ribosomal peptide synthesis (NRPS) has been noted in the biosynthesis of many small peptides in both bacteria and eukaryotes. The size and complexity of the enzyme machinery needed to catalyse the synthesis of surfactin means that conventional biochemical assays cannot give us any insight into the physiological state of the cells we are growing, so instead we are using QRT-PCR to monitor the transcription of key surfactin genes. The rapidity and accuracy of QRT-PCR allow us to monitor growth in bioreactors, especially important as we are developing a continuous method of production.

Although our QRT-PCR methodology is fairly straightforward (two to three genes of interest, plus three housekeeping genes give relative expression levels) we are hampered by several factors. Firstly, most of the research team are chemical engineers rather than molecular biologists, so our reagents must be simple to use and come with clear instructions. Secondly, we require a great dynamic range, since we monitor expression of genes such as *srfAA* from effectively zero to being one of the most numerically dominant mRNA molecules in any sample. Thirdly, we try to collect data every hour for between 12 and 36 hours, in fact as long as the lead postdoc can stay awake. Gene expression is not the only measurement that is taken, so the assay must allow quick error checking. Lastly, we do not have high-throughput QRT-PCR facilities: all the assays have to be set up manually before analysis on the ABI 7900.

Before settling on Thermo Scientific reagents for QRT-PCR, we tried a variety of different manufacturers’ reagents. The easiest to use were Thermo Scientific Verso™ QRT-PCR master mixes. The reproducibility and dynamic range were comparable to or better than reagents from other manufacturers (including those that came with the QPCR machine), but what really sets them apart is the blue colouration. This means that under time pressure, any operator, even the most inexperienced, can set up the reactions in 96-well plates confidently. As the volumes are small (25 microlitres) it is also possible to check by eye whether all the components have been added, particularly useful given the numbers of different primers used in a single plate. Most of our work has been done using SYBR Green I, but we have obtained comparable results using corresponding master mixes for probe detection too.
A further improvement in ease of use was the recent introduction of the white Thermo Scientific ABgene® SuperPlate™ (Figure 3). We tried them to see by how much the white colour would improve assay sensitivity. We found that assay sensitivity improved even at very low template concentrations. Pipetting clear reagents into white plates is difficult, which increases the likelihood of introducing errors. This may cause more handling problems than can be justified by the increase in sensitivity. However, the blue-coloured Thermo Scientific Verso QRT-PCR reagents enabled us to spot our pipetting errors before they impacted our results. Consequently, we were able to develop an extremely reliable assay.

In summary, the combination of Thermo Scientific Verso QRT-PCR master mixes and white Thermo Scientific ABgene SuperPlates have proved a high-performance, cost effective addition to the research effort of our laboratory. We hope to exploit the system further by using Thermo Scientific reagents to determine the absolute numbers of bacteria in our fermenters and foams.

PCR Mycoplasma test kit
Ready-to-use PCR Mix for the detection of Mycoplasma in Cell Culture

The PCR Mycoplasma Test Kit is designed to detect the presence of mycoplasma contaminating biological materials, such as cultured cells. Mycoplasma detection by the direct culture procedure is time-consuming and some mycoplasma species are difficult to cultivate. Using PCR testing, results are obtained within a few hours, since the presence of contaminant mycoplasma can be easily detected simply by verifying the bands of amplified DNA fragments in electrophoresis. There is no need to prepare probes labelled with radioisotopes, or to calculate enzyme, dNTP’s or buffer concentrations. Instead, a ready-to-use, optimised PCR mix is supplied. The primer set allows detection of various mycoplasma species (M. fermentans, M. hyorhinis, M. arginini, M. orale, M. salivarium, M. hominis, M. pulmonis, M. arthritidis, M. bovis, M. pneumoniae, M. pirum and M. capricolum), as well as Acholeplasma and Spiroplasma species, with high sensitivity and specificity.

Principle
rRNA gene sequences of prokaryotes, including mycoplasmas, are well conserved, whereas, the detection procedure utilising the PCR process with this primer set consists of:
1. Amplification of a conserved and mycoplasma specific 16S rRNA gene region using two primers.
2. Detection of the amplified fragment by agarose gel electrophoresis.
This system does not allow the amplification of DNA originating from other sources, such as cultured cells or bacteria, which affect the detection result. Amplification of the gene sequence with PCR using this primer set enhances not only the sensitivity, but also the specificity of detection. Amplified products are then detected by agarose gel electrophoresis.

Protocol
A. Test sample preparation
Transfer 0.5 – 1.0 ml cell culture supernatant into a 2 ml centrifuge tube. To pellet cellular debris, centrifuge the sample at 250 x g briefly. Transfer the supernatant into a fresh sterile tube and centrifuge at 15 000 - 20 000 x g for 10 minutes to sediment mycoplasma. Carefully decant the supernatant and keep the pellet (the pellet will not always be visible). Re-suspend the pellet with 50 µl of the Buffer Solution and mix thoroughly with a micropipette. Heat to 95 °C for 3 minutes. The test sample can be stored at this stage at –20 °C for later use.

B. PCR amplification
1. Prepare the reaction mixture in a PCR tube by combining the reagents shown below:

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₂O</td>
<td>35 µl</td>
</tr>
<tr>
<td>Reaction Mix</td>
<td>10 µl</td>
</tr>
<tr>
<td>Test sample</td>
<td>5 µl</td>
</tr>
</tbody>
</table>

2. Overlay mineral oil (approximately 40 µl) to avoid the evaporation of the reaction mixture.
3. Place all tubes in DNA thermal cycler. Set the parameters for the following conditions and perform PCR:

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>94 °C</td>
<td>30 secs</td>
</tr>
<tr>
<td>94 °C</td>
<td>30 secs</td>
</tr>
<tr>
<td>60 °C</td>
<td>120 secs</td>
</tr>
<tr>
<td>72 °C</td>
<td>60 secs</td>
</tr>
<tr>
<td>94 °C</td>
<td>30 secs</td>
</tr>
<tr>
<td>60 °C</td>
<td>120 secs</td>
</tr>
<tr>
<td>72 °C</td>
<td>5 min</td>
</tr>
</tbody>
</table>

35 cycles

Kit Components
1. Reaction Mix: 200 µl
2. Buffer Solution: 1 ml
3. Positive Template Control 20 µl

Reagents not supplied
1. Mineral Oil
2. Agarose gel
3. Distilled sterilised water

Equipment required
- Authorised thermal cycler for PCR
- Microcentrifuge tubes
- Agarose gel electrophoresis apparatus
- Microcentrifuge
- Micropipettes and pipette tips (autoclaved)

Storage: -20°C
Avoid repeated changes in the Reaction Mix temperature.
When in use, always keep the Reaction Mix on ice!


lengths and sequences of the spacer region in the rRNA operon (for example the region between 16S and 23S gene) differ from species to species. The
C. Analysis of amplified products by gel electrophoresis
1. Apply 20 µl of the PCR product to the gel electrophoresis.
2. Perform agarose gel electrophoresis with the PCR amplified samples to verify the amplified product and its size. Use 2% agarose gel.

The size of DNA fragments amplified using the specific primers in this kit is 270 bp.

Note: When the system is very sensitive, annealed primer or primer dimers might be detected as smaller bands (20 - 50 bp).

D. Control Template
By the use of 1 µl of Positive Template Control as a test sample, PCR efficiency can be checked.
The size of the PCR product obtained using the positive template with primer pairs is 270 bp.

Treatment of Mycoplasma-infected cells with antibiotics
Myco-1 (A5222), Myco-2 (A5233) and Myco-3 (A5240)

The contamination of cells with mycoplasma is a very common problem, even though it often goes unnoticed as no cloudiness appears in the cell culture. Nevertheless the contamination often causes biochemical changes as well as changes to the immunological properties of the cells. Since mycoplasma infected cells cannot always be discarded, many complicated methods have been suggested for the elimination of the mycoplasma.

AppliChem is now offering a combination of antibiotics, which have been shown to be effective in the elimination of mycoplasma species that account for 90% of the contamination found in cell culture. When used according to the following instructions, no cytotoxic effects will occur. Store Myco solutions at –20 °C. Several freeze / thawing cycles will not affect the quality.

Myco-1 and Myco-2
Myco-1 is based on the antibiotic Tiamulin, which is produced by the fungus Pleurotus mutilus. Myco-2 is based on Minocycline, a Tetracycline derivative. Myco-1 (A5222) and Myco-2 (A5233) are generally used sequentially in combination.

Instructions for use:
1. Do not use the two solutions together, rather sequentially!
2. Add 1 ml Myco-1 to 100 ml medium, and maintain the contaminated cells in this mixture for 4 days. Any fresh medium added should also contain Myco-1.
3. After 4 days, add 1 ml Myco-2 to 100 ml fresh medium, and maintain the cells in this second mixture for 3 days.
4. The above, together, are considered as one treatment cycle. it may be necessary to repeat this cycle 2-3 times.
5. During the process, the cells can be tested for mycoplasma contamination, and results can then be used to shorten the process when possible.

Myco-3
Myco-3 (A5240) is based on the Ciprofloxacin antibiotic, which is a member of the fluoroquinolone group. Many mycoplasma species have been found to be sensitive to Myco-3, including A. laidlawii, M. orale, M. hyorhinis, M. fermentans and M. arginini. These species are responsible for most of the contamination in cell culture. At the concentrations recommended for use (~1 µg/ml), no cytotoxic effects have been found, and the treatment is quite easy to perform. The pH-value of the solution is 4 - 5. When added to medium the pH change is negligible.

Instructions for use:
1. Add 1 ml Myco-3 to 100 ml medium.
2. Continue the treatment for a total of 14 days, while changing the medium (containing Myco-3) every 2-3 days.
3. Retain the cells in the growth medium for additional 14 days before re-testing for mycoplasma. Sometimes the treatment reduces the mycoplasma concentration without eliminating them and they will grow again after the antibiotic treatment. If this is the case, treat another time with the antibiotics Myco-1 and Myco-2.

Literature
Platform technologies are one approach toward standardising and streamlining manufacturing operations for personalised treatments. Influenza vaccine, for example, is developed and manufactured rapidly, if inelegantly, through well known platform techniques involving egg-based virus culture. For treatments derived from a patient’s own cells, an ideal cell culture platform approach should be:

- Compact
- Rapidly set up
- Composed of single use disposable elements
- Closed and self-contained
- Automated

In 2001, Biovest International began investigating options for manufacturing patient-derived therapies in an automated compact cell culture device that required significantly less expertise and human interaction than traditional stainless steel or multiple flask-based cell cultures.

Biovest design was driven by ongoing development of a vaccine against non-Hodgkin’s lymphoma, which is currently in Phase III testing at several sites. The challenge was to develop a scalable, commercial platform technology that allowed multiple instruments to be run simultaneously in the same suite. Furthermore, this production methodology needed to be suitable for production in a cGMP facility in which multiple patient-derived products are manufactured simultaneously.

Biovest recognised early on that no such personalised biologic could be commercialised without a significant reduction in process complexity compared with conventional cell culture. Commercial application of a personalised treatment would never be a reality if the expertise and attention required a PhD biologist or senior engineer to conduct these cell culture processes. Similarly, Biovest believed that regulators would never approve such a device unless concerns about cross-contamination, or technician error, among multiple, simultaneously operating systems, were addressed in the instrument design.

Necessity, the mother of invention

In the mid-1980s, through its predecessor company, Cellex Biosciences, Biovest was the first vendor to offer commercial-scale hollow fibre bioreactor systems. Biovest’s large array of instruments and systems already provided hollow fibre technology suitable for research to pharmaceutical production scale.

In 1999, the US National Cancer Institute (NCI) promulgated a contract for producing antibodies from patient-specific cell lines. More specifically, NCI had developed a personalised immunotherapeutic approach to treat people with low grade, follicular lymphoma. This approach quickly moved into clinical trials, followed shortly by transfer of ownership to Biovest.

As the corporate sponsor of the project, Biovest began designing a novel production device that could be used in a multi-product facility to facilitate the manufacture of patient-specific products under U.S. Food and Drug Administration GMP guidelines.

Biovest’s personalised lymphoma vaccine product(s) progressed through clinical testing, the deficiencies of conventional cell culture manufacturing became evident, promoting a complete re-thinking of the production process. It became clear that the process ideally required a device that could offer sample containment or segregation, high-throughput, small-footprint, and ability to concurrently manufacture thousands of patient-specific products in one facility. Based on this premise, the AutovaxID™ emerged after several years of development effort that combined cell culture, engineering, electrical and mechanical expertise.
The AutovaxID™ is a self-contained, completely enclosed, fully automated hollow fibre bioreactor that permits rapid, efficient scaleup of patient-derived cells. Because it is based on hollow fibre bioreactor technology, the AutovaxID™ device is an ideal system for high-density cell culture and production of monoclonal antibodies or other therapeutic proteins.

Capabilities

AutovaxID™ is well suited for growing any antibody-secreting cell line, including hybridomas and Chinese hamster ovary (CHO) lines, currently the workhorses of commercial therapeutic protein manufacture. In the case of our lead product, BiovaxID, patient-specific vaccines are created by fusing patient-derived tumour B-lymphocytes with an established murine heterohybridoma cell line. Since B lymphocytes, by themselves, are somewhat difficult to culture and poor ex vivo producers of monoclonal antibodies, fusion with another hybridoma line provides them with enhanced ability to proliferate and secrete their unique immunoglobulin. This immunoglobulin (IgG or IgM) is unique to each clonal cell population, and in this case, represents a unique tumour antigen. More specifically, the idiotype, or CD3 region of these immunoglobulins is unique to each patient. As such, fusing cancerous B cells with an established hybridoma line gives us the ability to manufacture large quantities of tumour-specific protein, which is purified and used to immunise the patient against recurrence of lymphoma.

The National cell culture centre routinely uses the AutovaxID™ for its monoclonal antibody production services. Antibodies have been produced from client derived cell lines that include mouse and rat hybridomas, human heterohybridomas, and transfected CHO lines. Production levels vary considerably, depending on the cell-specific productivity and intended duration of each culture. Overall, quantities produced ranged from 0.5 g to 5.6 g in 45 days. In general, an average hybridoma that produces 15-25 µg/ml in T flasks can be expected to produce 1-2 g of antibody in 30 days from the AutovaxID™. More importantly, these cultures required approximately 75% less labour than that typically required for conventional hollow fibre bioreactor production instrumentation (10-15 hours/month, as opposed to 40-45 hours/month). As a result, technician time was more available for additional projects, which significantly increased overall productivity of the laboratory.

Looking under the hood

The AutovaxID™ instrument consists of two components: the control unit and an enclosed, disposable bioreactor (cultureware). The disposable component is completely enclosed in a plexiglass housing that slides into, and snaps in place within the instrument (see figure above). With conventional hollow fibre instruments, technicians must first prepare the cultureware for installation into the instrument. This may include loading peristaltic pumps, aseptically inserting an autoclaved pH probe, and manually opening and closing clamps to facilitate a preinoculation fill/flush procedure. The entire preparative process may take several hours and is prone to mistakes with an inexperienced technician. All these manual operations have been eliminated with the AutovaxID™. Technicians need only snap the cultureware into the device and connect a media feed source. Following an automated fill/flush procedure, inoculation and pH calibration, the instrument’s process control then allows for unattended operation for the duration of the culture.

The disposable cultureware for the AutovaxID™ contains of fluid or product-contact components, including the bioreactor and associated tubing, pH probe, cycling and harvest bags, and a gas exchange cartridge to oxygenate and provide feedback control of pH in these densely packed cultures.

The AutovaxID™ control unit contains a built-in computer, touch screen display and built-in solid-state refrigerator. The entire system can be monitored and controlled remotely via a web browser. The software was designed to maintain process parameter setpoints for pH, lactic acid, and temperature, and also to monitor pump speeds and calculate media volumes. When media reaches a critical low point, for example less than 5 litres, the technician is alerted to replace or add a new container. Finally, the AutovaxID™ instrument was designed with software features, such as password protection and security required for production in a regulated setting.

The AutovaxID™ was specifically designed for use in multi-product facilities that must manufacture large numbers of patient-specific, cell-based therapies simultaneously and in a relatively compact space. In designing the AutovaxID™ to include such features as product segregation capability, automation, and software security Biovest believes that the company has addressed key regulatory issues relating to production of personalised biologics by the instrument.

AutovaxID™ has shown its value and versatility for producing patient-specific secreted proteins for therapeutic use, at a cost that makes potentially life saving therapies accessible to patients with many serious illnesses. While Biovest expects to continue to serve this important area of personalised medicine, Biovest also expects that many new and promising treatments will arise from cell-based therapies. Biovest is currently exploring ways to optimise the AutovaxID™ for applications that involve recovery of whole cells grown in the AutovaxID™ for therapeutic use. At present, our challenge is to develop a process that allows for cost effective growth and efficient recovery of unique or specialised cell types, including stem cells.
Pall Life Sciences – Sterile filtration range

Race through your research, not your budget

While there is no substitute for aseptic technique, sterilisation of liquids used during cell culture assures you that media and reagents are not a source of contaminants. Heat sterilisation (autoclaving) is not an option for many liquids, as critical components such as proteins are heat labile. Filtration, therefore, is the method of choice for sterilising cell culture media and additives. Pall manufactures membrane filter materials with high throughputs and low extractables to produce devices suited for a wide range of applications. Whether you are preparing small volumes of reagents, individual bottles of buffers or media, or developing pharmaceutical processes, we have the products to meet your sterile filtration needs.

Stringent requirements for cell culture sterilisation

At Pall, quality and ease of use are engineered into every device. We manufacture our own membranes to ensure consistent performance from lot to lot. Examples of our stringent quality specifications include:

- **Cell culture certification**: products with 0.2 μm membranes are certified for use in cell culture applications. Representative samples of these products are tested for retention of a bacterial challenge with *B. diminuta*
- **Sterilisation by gamma irradiation** to eliminate potential cytotoxic residuals associated with EtO sterilisation. These presterilised devices are individually packaged for convenience
- **Biological safety**. Products pass USP Class VI-121 °C plastics tests, a stringent set of biological safety tests for manufactured plastics devices
- **Non-pyrogenicity**. Products are tested for bacterial endotoxin using the Limulus Amoebocyte Lysate (LAL) test to ensure safety
- **Low extractables**. Products are optimised to reduce extractables, ensuring that unwanted materials are not introduced into filtered liquids

Fast, final filtration and high throughputs with Supor membrane

Pall’s key membrane for all your sterile filtration requirements is the Supor (PES) membrane. Supor membrane is expressly formulated and optimised for biological, pharmaceutical and sterilising filtration requirements. Supor membrane gives high flow rates and low protein binding.

The Supor membrane is available in a variety of sterile devices to suit your filtration needs, Supor Acrodisc syringe filters for small volume filtration through to VacuCap vacuum filtration devices for mid volumes and then AcroPak™ capsule filters for larger volumes.
VacuCap & VacuCap PF Vacuum Filtration Devices

Innovative bottle-top filters for fast vacuum filtration of 100 ml to 5 l of aqueous solutions.

The VacuCap innovative bottle-top filters eliminate the possibility of contamination from transfer steps. Liquid is drawn directly from the mixing reservoir and filtered directly into the desired container of choice.

- Contains the low protein binding Supor membrane (hydrophilic polyethersulphone) for superior flow rates and high throughputs
- Match filter device size to sample volume by choosing from two sizes
- Available built-in prefilter increases throughput of viscous or particulate-laden solutions, such as serum containing media
- Patented small design accepts a variety of collection vessels and reduces both storage space and waste
- Reduce mycoplasma with the 0.1 μm pore size device

Introducing the AcroPak capsule family of products

For filtration of larger volumes of liquid, Pall have a whole family of sterile capsule filters ranging from 200 cm² EFA (effective filtration area) to 1500 cm² EFA devices.

- Ensure sterile, mycoplasma-free cell culture media with AcroPak capsules with 0.1 μm Supor® membrane
- Provide higher throughputs and faster flow rates than similar-size competitive devices.
- 100% integrity tested to assure sterile filtrate
- Fusion-welded components eliminate the potential for release of extractables from sealing adhesives
- Ideal for solutions where low protein binding is required
- Upstream air vent prevents vapour lock

The AcroPak capsules contain Pall’s patented Supor for unsurpassed speed and extremely low binding, therefore you’ll use less time, spend less money on devices, and get more consistent results.

<table>
<thead>
<tr>
<th>Description</th>
<th>Qty/Pk</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>VacuCap 60 vacuum filtration device, 0.1 μm, 60 mm sterile</td>
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</tr>
<tr>
<td>VacuCap 60 vacuum filtration device, 0.2 μm, 60 mm sterile</td>
<td>10</td>
<td>515-0017</td>
</tr>
<tr>
<td>VacuCap 60 vacuum filtration device, 0.45 μm, 60 mm sterile</td>
<td>10</td>
<td>515-0018</td>
</tr>
<tr>
<td>VacuCap 60 vacuum filtration device, 0.2 μm, 60 mm sterile, (supplied with individually attached tubing for each filter device)</td>
<td>10</td>
<td>516-9813</td>
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<tr>
<td>VacuCap 90 vacuum filtration device, 0.1 μm, 90 mm sterile</td>
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<td>515-0012</td>
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<td>VacuCap 60 PF vacuum filtration device, 0.8/0.2 μm, 60 mm, sterile</td>
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</tbody>
</table>
NUNC Brand has introduced a new and broader range of their NUNC polycarbonate Cell Culture Inserts. The range now offers 4 sizes of polycarbonate inserts for use with Multidishes 24, 12 and 6, as well as a small version for use with Multidish 6.

All inserts are offered with 3 different membranes: a 0.4, 3 or 8 µm pore size polycarbonate membrane treated for cell culture. The inserts are packed in Multidishes for easy access/usage, sterile SAL 10⁻⁶, endotoxin-free, surface treated for optimal cell attachment and packed in re-sealable bags. Also, they have tabs for better and secure manual handling.

**Cell surveillance simplified – easy cultivation of most cell types without matrix coating**

**Benefits**

**Benefits of the polycarbonate membrane**
- Useful for electron microscopy, where Anopore™ is too electron dense
- Membrane can be removed from support using a sharp blade
- Pre-packed in Multidishes

**Benefits of the Anopore™ membrane**
- Inorganic membrane provides a rigid, uniform growth surface with controlled pore size and distribution
- Unique optical clarity when wet for easy microscopic evaluation
- Unique chemical properties allow the use of a wide range of fixation and staining techniques

**Common benefits of polycarbonate and Anopore™ membranes**
- Support the attachment and growth of most cell types without matrix coating
- Exhibit low non-specific binding during staining
Both membranes are specially treated to provide a surface that is excellent for cell attachment and growth. The Anopore™ membrane is a rigid inorganic membrane that is highly transparent when wet and highly porous (40-50% surface porosity). This membrane provides maximum clarity for microscopy, is non-autofluorescent, and highly resistant to most solvents. Some typical applications are epithelial polarisation studies, transport studies, toxicity testing and electron microscopy.

The polycarbonate membrane does not have the transparent characteristics of the Anopore™ membrane. Due to the larger pore sizes available with the polycarbonate membrane, it is particularly suitable for invasion and chemotaxis studies. These membranes are easily removed and are ideal for sectioning for transmission electron microscopy using a glass knife.

Anopore™ is a trademark of Whatman Scientific, Ltd.

Support a wide range of cell lines

NUNC Cell Culture Inserts have been shown to support a wide range of cell lines, in the absence of matrix coating, but they are fully compatible with all standard coating techniques. Extensive independent trials have shown NUNC Cell Culture Inserts to be ideally suited to a wide range of applications. A technical bulletin is available, contact your VWR Life Sciences specialist.

Cells cultured on uncoated NUNC Inserts include:

<table>
<thead>
<tr>
<th>Cell Lines</th>
<th>BHK</th>
<th>CACO-2</th>
<th>HeLa</th>
<th>WRC</th>
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<tbody>
<tr>
<td>Hep-G2</td>
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<td>L929</td>
<td>CHO</td>
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<tr>
<td>LLC-PK</td>
<td>MDCK</td>
<td>3T3</td>
<td></td>
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</tr>
</tbody>
</table>

Primary cells

Chick Embryo Endothelial Cells, Human Kidney Epithelial Cells, Bovine Corneal Endothelial Cells, Human Umbilical Cord Endothelial Cells, Rat Hepatocyte, Human Retinal Pigment Epithelial Cells

Application examples:

Pore sizes

Transport studies

- 0.4; 3.0 µm
  - Ions, hormones, growth factors, etc.
  - Drug effects on vascular endothelial permeability
  - Drug transport across epithelial and endothelial barriers
  - Drug transport across brain microvascular endothelial cells

Chemotaxis studies

- 3.0; 8.0 µm
  - Migration of eosinophils, neutrophils, macrophages, etc.

Invasion studies

- 3.0; 8.0 µm
  - Tumor invasion and metastasis
  - Invasion inhibitors
  - Extracellular matrix effects

Co-cultivation studies:

- 0.4; 3.0 µm
  - Cell-cell interaction via soluble factors

Tissue engineering:

- 0.4; 3.0 µm
  - Angiogenesis
  - Dermal/epidermal and epithelial tissue models
Taking monolayer cell culture from research to production

- Multi-language display prompts - select from English, French, German, Spanish, Italian and Portuguese
- Now available with 5 or 8 position decks
  - 88 position fixed frame production model
  - 55 position fixed frame production model (fits our roll-in incubator)
  - 5 position to 45 position modular spaced unit allows flexibility of adding or removing decks
- Programmable rotation direction and soft start/stop
- Built-in rotation alarm
- Accurate digital display of bottle RPM
- CART₂ ready!
- Optional battery backup
- Optional temperature monitoring
- Meets CE and CSA standards

As an alternative to T flasks, Wheaton offers cell culture roller apparatus which uses roller bottles which provide maximum surface area for confluency. The entire surface of a roller bottle is used to grow monolayer cells without having to fill the bottle with expensive media. The roller method provides a much larger surface area than the static method.

Roller Cell Culture Overview

There are several advantages of roller culture over static monolayer culture:

1. Increased surface area in a single container providing larger cell yields
2. Increased ratio of cell growth surface to container volume allows increased rate of gas exchange
3. Simplified scale up from research to production without changing protocols

The amount of medium and cells in suspension added to the bottle is relatively small compared to the total volume of the bottle and is usually based on the cell density required. The atmosphere of the bottle is often purged at 5% CO₂ to increase gas exchange efficiency and help stabilise pH.

The initial rotation speed of the bottle is usually very slow (<1 rpm) to allow the cells to attach and begin to proliferate. The speed is often increased later. The optimum speeds are different for different cell lines and bottle diameters. Bottle diameter and length determine the surface area on which cells can grow. The larger the bottle, the greater the inner surface area. It also affects the speed range. For a given roller apparatus speed, larger diameter bottles will rotate more slowly than small diameter bottles. Therefore a roller apparatus allowing programming of the bottle diameter and the desired bottle rotation speed may be desired.

The Wheaton R₂P roller apparatus provides this capability.

Roller bottles are sometimes removed from the roller apparatus to feed with fresh medium. The bottle is taken into a sterile area where the media is carefully aspirated not disturbing the cells and fresh media is dispensed. This process is often performed using a peristaltic pump such as the Wheaton Omnispense Plus (see last edition of the VWRbioMarke magazine).

To harvest cells, the medium is aspirated and the cells are usually rinsed with a 1x Phosphate buffered saline solution (PBS). Trypsin or Cell Dissociation Buffer is added to remove the connective tissue and allow the cells to be detached from the bottle, then drained and fresh media is added.

Conclusion

Wheaton Roller Apparatus such as the R₂P allows flexibility for scale-up and production of monolayer cell cultures using roller bottles. Wheaton Roller Apparatus enables easy scale-up for anchorage-dependent cell lines by utilising increased surface area, gentle agitation and improved gas exchange.
Increased cell yields using Wheaton roller apparatus

As an alternative to T flasks, Wheaton offers cell culture roller apparatus which uses roller bottles which provide maximum surface area for confluency. The entire surface of a roller bottle is used to grow monolayer cells without having to fill the bottle with expensive media. The roller method provides a much larger surface area than the static method.

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3. Simplified scale up from research to production without changing protocols

The amount of medium and cells in suspension added to the bottle is relatively small compared to the total volume of the bottle and is usually based on the cell density required. The atmosphere of the bottle is often purged at 5% CO₂ to increase gas exchange efficiency and help stabilise pH.

The initial rotation speed of the bottle is usually very slow (<1 rpm) to allow the cells to attach and begin to proliferate. The speed is often increased later. The optimum speeds are different for different cell lines and bottle diameters. Bottle diameter and length determine the surface area on which cells can grow. The larger the bottle, the greater the inner surface area. It also affects the speed range. For a given roller apparatus speed, larger diameter bottles will rotate more slowly than small diameter bottles. Therefore a roller apparatus allowing programming of the bottle diameter and the desired bottle rotation speed may be desired. The Wheaton R₂P roller apparatus provides this capability.

Roller bottles are sometimes removed from the roller apparatus to feed with fresh medium. The bottle is taken into a sterile area where the media is carefully aspirated not disturbing the cells and fresh media is dispensed. This process is often performed using a peristaltic pump such as the Wheaton Omnispense Plus (see last edition of the VWRbioMarke magazine).

To harvest cells, the medium is aspirated and the cells are usually rinsed with a 1x Phosphate buffered saline solution (PBS). Trypsin or Cell Dissociation Buffer is added to remove the connective tissue and allow the cells to be detached from the bottle, then drained and fresh media is added.

Conclusion

Wheaton Roller Apparatus such as the R₂P allows flexibility for scale-up and production of monolayer cell cultures using roller bottles. Wheaton Roller Apparatus enables easy scale-up for anchorage-dependent cell lines by utilising increased surface area, gentle agitation and improved gas exchange.
Preparation of BD Falcon™ cell culture inserts for confocal indirect immunofluorescence

Fixation and staining of Caco-2/bbe (C2) cells with various dyes

BD Falcon™ cell culture inserts feature smooth, transparent, microporous polyethylene terephthalate (PET) membranes that provide an ideal substrate for culturing a wide variety of cell types. These inserts are ideally suited for most histological procedures, as they can be processed intact, using standard techniques, by passing them through a series of fixation and staining solutions. The insert membrane, which offers excellent chemical resistance to organic solvents, can be easily cut with a razor blade or scalpel in order to remove samples for embedding, sectioning or staining.

BD recommends the following procedure for the preparation of BD Falcon™ Cell Culture Inserts for confocal indirect immunofluorescence:

1. Grow cells on BD Falcon™ cell culture inserts for 6-well plates (Cat. No. 734-0032), and perform required experimental treatment(s) of cells.
   **NOTE:** Buffer should be added to both sides of the insert for all incubations. All volumes, except as noted, are 1,5 ml in the apical chamber and 2 ml in the basolateral chamber, per insert/well in the 6 well plate.

2. Aspirate the medium, then add K-PIPES buffer containing 3,75% formaldehyde at RT. Incubate 5 min at RT.

3. Aspirate K-PIPES containing formaldehyde. Add NaBO₄ buffer containing 3,75% formaldehyde. Rotate gently and fix 10 min at RT.

4. Aspirate, and wash with PBS. Replace with fresh PBS and incubate 5 min at RT.

5. Aspirate, and then permeabilise by adding PBS/TRITON. Incubate 15 min with gentle rotation at RT.

6. Aspirate PBS/TRITON and replace with blocking solution comprised of PBS/BSA. Perform three incubations/washes (5 min each). Add the appropriate dilution of primary antibody to the top (apical) and underside (basolateral) of the cell culture insert membrane in the following manner:
   - Cut a circle of parafilm to fit the well (two pieces will be needed for each well).
   - Place 50 µl of primary antibody diluted in PBS/BSA onto the top surface of the membrane and cover with a parafilm circle.
   - Turn the insert over to repeat for the underside of the membrane.
   - Add 50 µl of diluted primary antibody to the underside of the membrane, and cover with a parafilm circle.
   - Keep in a humidified box at 4 °C overnight.

7. Remove the parafilm and wash BOTH sides of the membrane 3x with PBS at RT. For each wash, incubate for at least 5 min.

8. Block the membrane with PBS containing appropriate serum (corresponding to the species used to generate the secondary antibody). Dilute the secondary antibody approximately 1:1000 in PBS and place on the apical and basolateral sides. Incubate 60 min at RT.

9. Wash 3x for 5 min with PBS. Add appropriate dilution of secondary antibody (can start with 1:1000) and place 50 µl on apical and basolateral sides. Cover with the parafilm circles (which can be reused) and incubate 60 min at RT.
   **NOTE:** If needed, a 1:1000 dilution of FITC- or Rhodamine-conjugated phalloidin can be added at this time to stain microfilaments.

10. Wash the membranes thoroughly with PBS. Perform at least three washes for 5 min each (additional washes may be required for some antibodies).

11. Place four small drops of nail polish on a glass slide to mark the location corresponding to the corners of a square No. 1 Coverslip. Remove the PET membrane from the insert housing with a razor blade or scalpel. Cut the edges off to prepare a square cut piece of membrane that is smaller than the square No. 1 Coverslip. Place the square cut membrane in the middle of the four spots of nail polish, cell side up, and place 38 µl of a solution of 50 mg/ml n-propyl gallate (nPG) directly onto the membrane. Place a square No. 1 Coverslip over the membrane to seal the area. Remove excess nPG from the sides, and then use nail polish to seal all of the edges. Allow the nail polish to dry, keeping the slides in a dark box at 4 °C. Perform imaging analysis as soon as possible.

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1Mark W. Musch, Ph.D., Margaret Mary Walsh-Reitz, Ph.D., and 2Marshall Kosovsky, Ph.D.
1The Martin Boyer Laboratories, Inflammatory Bowel Disease Research Center, Department of Medicine, The University of Chicago, Chicago, IL, USA; 2BD Biosciences – Discovery Labware, Billerica, MA, USA.
Figure 1. Confocal micrographs of Cy2-tagged α-subunit of Na+-K+-ATPase in Caco-2/bbc (C2) cells. Cells were treated for 60 min with 0.6 mM NH2Cl alone or after 1-hr treatment with phalloidin (100 µM), or with the actin destabiliser cytochalasin D (2 µM). Distribution of the α-subunit of Na+-K+-ATPase was assessed by confocal imaging. Apical slices (xy, en face images) are shown in the left. Vertical stack slices (xz) from regions marked with white dashed lines are shown in the middle, and a portion of this is enlarged to the right. Lines show representative xz reconstructions (n>4 from each panel) obtained from a series of xy slices (0.2 µm apart).

The Na+-K+-ATPase α-subunit is pseudo-coloured green. The red colour is transmitted reflected light used to monitor monolayer integrity. Arrows on apical membranes of monolayers indicate the apical movement of the α-subunit.

As can be observed, a large majority of the ATPase subunit appears at the outer plasma membrane. This ATPase is not localised to the apical pole in untreated monolayers; however, after NH2Cl treatment, green staining appeared at the apical pole (middle and right).

Materials and Methods

Solutions

- PBS mixture (1 mM CaCl2 and 1 mM MgCl2).
- PBS/TRITON mixture (1 mM CaCl2, 1 mM MgCl2, and 0.2% (v/v) TRITON™ X-100).
- PBS/BSA mixture (1 mM CaCl2, 1 mM MgCl2, and 1% BSA).
- K-PIPES fixation buffer (80 mM K-PIPES, 5 mM EDTA, 2 mM MgCl2, pH = 6.5).
- NaBO4 fixation buffer (100 mM NaBO4, pH = 11.0).
- N-propyl gallate (nPG): dissolve 500 mg of nPG in 7 ml glycerol (do not use heat). Increase volume to 10 ml with PBS. Add 163 mg of TRIS base to ensure that pH is >8.5 (additional TRIS base can be added if necessary). Store at -20°C in dark.

References

Thermo Scientific Multidrop Combi
Ready for any dispensing challenge

Offering unrivalled levels of performance, versatility and simplicity, pharmaceutical and biotech laboratories will find it more than meets their expectations. Based on the proven and highly reliable Multidrop range, Multidrop Combi has been designed for effortless high speed dispensing of different types of solutions. For high throughput screening Multidrop Combi dispenses into 1536 well plates at nanolitre levels with minimal dead volume. At the other end of the scale, being able to dispense at volumes up to 2500 µl offers biotech researchers a wide range of applications. Add in full robotic capability and the Multidrop Combi is your answer for problem-free, high throughput bulk dispensing.

Total flexibility
From 6 to 1536 wells, the Multidrop Combi is capable of dispensing into a full range of plates and with automated dispensing height adjustment for low profile to deep well plates, it offers complete flexibility for any dispensing application. The small footprint enables Multidrop Combi to fit into laminar cabinets and together with the extensive dispensing volumes, versatile column selection and dispensing speed options, the Multidrop Combi is ideal for all pharmaceutical and biotech researchers.

Precise dispensing
Multidrop Combi uses the peristaltic pump technology and unique Multidrop dispensing cassettes to ensure absolute precision across an unrivalled volume range. CV’s of <10 % at 0,5 µl will empower drug discovery teams to design new high throughput assays on 1536 well plates (Figure 1). Similarly, dispensing up to 2500 µl is ideal for some delicate cell-based assays. Dead volumes are minimised at all settings. A wide range of dispensing speeds is also possible and up to 8 different solutions can be dispensed simultaneously. The possibility to adjust different volumes for each individual column enables the user to fill the plate with different dilutions of the same reagent. Overall result? A steady flow of highly reproducible, meaningful data leading to real increases in productivity.
Easy to use

Through the simple and visual interface, Multidrop Combi enables users to take full advantage of its technical advances. Descriptive icons make it easy to change all dispensing parameters for customised work - such as pump speed, tip height, offset values, dispensing direction etc - or go straight to pre-set protocols (Figure 2). The user can also store their own protocols in the instrument memory from which they can be launched in seconds. In all cases, the intuitive interface means little or no training is required, so productivity is raised.

Autoclavable dispensing cassettes and reagent back-flushing

To ensure sterile conditions and avoid cross contamination, the Multidrop Combi uses 8-channel detachable and autoclavable dispensing cassettes that are standard across the Multidrop range. Cassettes are selected according to the dispense volumes and user preference, and changed in seconds. Back-flushing of tubing and the Multidrop Combi’s negligible dead volumes minimises expensive reagent loss.

Full robotic compatibility

Multidrop Combi can increase productivity as a stand-alone instrument. However, when users take advantage of its full robotic compatibility, the real benefits become clear. The dispenser’s plate carrier is optimised for easy plate access by robotic arm grippers, linked via either the RS-232 serial or USB port. Furthermore, the Thermo Scientific RapidStak™ stacker can be added to create a complete benchtop solution for automated bulk reagent dispensing (Figure 2).
Nanolitre volume dispensing

**Thermo Scientific Finnpipette® Novus 5-50 µl**

The Finntip® Pocket reduces reagent costs through miniaturization of assays. It is suitable, for example, for diluting PCR primers. By filling the moulded pocket inside the tip with a specified amount of compound suspended in DMSO, it is possible to dispense nanolitre volumes with hand-held pipettes. The Finntip® Pocket is designed for use with the electronic Finnpipette® Novus 5-50 µl. The pipette can be programmed for different steps of dispensing with Finntip® Pocket.

**Finnpipette® Novus programming**

**Program 1: Loading the pocket**
- Select one of the nine programme settings places and PIPETTE mode.
- Select volume 17 µl and speed 3 for both aspiration and dispensing.

**Program 2: Rinsing the tip**
- Select programme settings and PIPETTE mode
- Select volume 5 µl and speed 5 for both aspiration and dispensing

**Program 3: Dispensing into the assay buffer**
- Select another PIPETTE mode
- Select volume 15 µl and speed 3 for both aspiration and dispensing

**Loading the pocket with source liquid**
1. Place Finntip® Pocket in the source liquid (compound in 100% DMSO).
3. **Press and hold the trigger** to dispense the excess back into the source liquid.
4. **Still holding the trigger**, remove the tips from the source liquid and blot them completely onto a clean absorbent tissue (empty the tip below the pocket)
5. Release the trigger.

**Rinsing the tip below the pocket**
1. Place Finntip® Pocket in the rinsing liquid (DI water).
2. Aspirate the rinsing liquid below the pocket using programme 2.
3. Dispense the rinse liquid back into the rinse liquid plate or reservoir.
4. **Still holding the trigger**, remove the tips from the rinse liquid and blot them completely onto a clean absorbent tissue.
5. Release the trigger.
6. Repeat steps 1-5 two to three times.

**Diluting and dispensing the source (compound) liquid**
1. Place Finntip® Pocket in the assay well filled with an aqueous assay buffer.
2. Aspirate 15 µl using programme 3. Make sure that the buffer upper meniscus passes by the pocket until the pocket contents are thoroughly mixed with the buffer.
3. Dispense the assay liquid back into the assay plate.
4. **Still holding the trigger**, remove the tips from the assay liquid.
5. Release the trigger.
6. Repeat steps 1-5 three to five times.

**Accuracy and precision testing of Finntip® Pocket**
The accuracy of Finntip® Pocket is ±15% (average of a rack of 96 tips). The CV should be below 6% across a rack of 96 tips.

**Materials**
- Oregon Green; prepare ~100 µM Oregon Green in 100% DMSO
- PBS buffer, pH 7.2
- Black 384 well Thermo Scientific Microtiter microplates (Cat. no. 735-0626) or other untreated black microplates
- Thermo Scientific Multichannel Finnpipettes (1-10 µl, 5-50 µl, 30-300 µl)
- Finntip® Pocket, Thermo Scientific Finntip® 10, Thermo Scientific Finntip® Flex 300
Preparing a standard curve

- Dispense 95 µl PBS buffer into the first column of a black 384 well microplate and 50 µl PBS buffer into the following eight columns.
- Add 5 µl of the ~100 µM Oregon Green solution to the first column, mix by pipetting up and down and transfer 50 µl to the next column.
- Continue transferring 50 µl to the adjacent columns.
  Note: Remove 50 µl from the last column to ensure that all wells contain 50 µl.
- Spin the microplate down for 1-2 minutes at 2000 rpm.
- Measure the fluorescence (RFU) using filter pair Ex 485/Em 538, for example, on Thermo Scientific Fluoroskan Ascent. Calculate the average RFU.
- The first column contains 2.5 µl Oregon Green/well, the next column 1.25 µl/well, etc.
- Prepare the standard curve by plotting the average RFU against the volume. Use only the smallest volumes, see Figure 1 for an example.

Testing of the Finntip® Pocket

- Dispense the same ~100 µM Oregon Green solution with Finntip® Pocket into 50 µl PBS buffer in a black 384 well Microtiter microplate (for example, into 32 wells).
- Spin the microplate down for 1-2 minutes at 2000 rpm.
- Measure the fluorescence as described above.
- Determine the precision of the RFU values: CV% = 100 * standard deviation/average. The actual volume can be determined from the standard curve. Accuracy is the difference between the dispensed volume and the volume of Finntip® Pocket (50 nl or 250 nl) and can be expressed as a relative value (acc%).

Finnpipette® Novus offers superior usability with Finntip® Pocket

Finnpipette® Novus is the leading brand of electronic pipettes, built and designed by extensive customer feedback. Each Finnpipette® Novus 5-50 µl has a Finntip® Pocket sample inside the pipette package. Advanced Novus features help in everyday laboratory routines with Finntip® Pocket and regular pipetting.
- Programmability: nine easy to use built-in memory slots for convenient routine pipetting
- Backlit display for high contrast
- Interface in six languages for comfort of use
- 2-year warranty with simple web registration
- Reliable performance with long lasting battery

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At one time or another most scientists working in the ‘biochemical’ sciences will have to dialyse something. Dialysis is a simple technique to exchange a solute’s solution or to separate/purify differently sized molecules. Macromolecules are dialysed by placing them in size-selective permeable tubing and subsequently equilibrating the sample with large volumes of new buffer: efficient dialysis relies upon appropriate selection of dialysis tubing and effective ‘washing’ that results from large volumes, multiple changes and full equilibration with the new buffer. I have recently been generating many fluorescent analogues of proteins and have used dialysis to remove ammonium sulphate from protein suspensions prior to labelling and to remove unincorporated succinimidyl esters of various fluorophores after labelling. For these steps I have been using Spectra/Por® dialysis tubing from Spectrum Laboratories®.

Spectra/Por® 1 – 7 dialysis tubing is made of regenerated cellulose (RC). Cellulose has long been used for dialysis as it is uncharged and does not readily absorb solutes. Further, the selectivity of cellulose membranes is not altered greatly by many chemicals or reasonable pH and temperature ranges. Processed cellulose has crystalline regions and these regions cross-link chains to introduce structural integrity to the cellulose. Depending upon how the cellulose is...
processed the number of crystalline areas varies and the resultant regions between the cross-links can act like size-selective pores.

Many Spectra/Por® Regenerated Cellulose (RC) basic membranes (Spectra/Por® 1, 2, 3, 4 and 5) come in 3.5, 6–8 and 12–14 kDa MWCOs and more expensive RC membranes (Spectra/Por® 6 and 7) have a range of MWCOs from 1–50 kDa. MWCO values are established by Spectrum® and represent the size at which a solute is 90 % retain during a test period. The small pore sizes available mean that relatively small molecules can easily be processed and I have used dialysis to buffer exchange samples of ~10 kDa with only minimal sample loss. Certain parameters such as pH may however alter MWCOs and so appropriate tubing needs to be established for a particular situation. That said, with my application using simple buffers the MWCOs have proven to be robust. Spectrum suggest that efficient separation can be achieved if the size ratio of the moieties is at least 25, this ratio is easy to achieve for buffer exchange of a protein or removal of unincorporated fluorescent dyes when labelling macromolecules. Equilibration of the membranes is relatively quick, especially for the larger pore sizes that I typically use, for instance, after labelling I can detect unincorporated dye molecules in my equilibration buffer within 2–4 hours during the first wash. This equilibration time means that 4 buffer changes can easily be performed in 24 hours. Spectra/Por® is supplied in an essentially ready-to-go format, no boiling in bicarbonate buffer is needed. Spectra/Por® 1–6 merely need soaking in distilled water for 30 minutes whereas other tubing is supplied pre-hydrated and must be stored at 4 °C. One concern is that these membranes contain trace amounts of heavy metals and sulphur although Spectrum® do also produce specific cleaning solutions and supply Spectra/Por® 7 which comes with minimal contaminants. Membranes can be sterilised and also have two year shelf lives. Finally, Spectra/Por® tubing is supplied in different widths depending upon the scale of dialysis you plan.

For me dialysis essentially provides a ‘low maintenance’ way to perform buffer exchange or purify a sample if there is a large size differential. Other methods do exist to perform the same task including ultra-filtration units that are faster and extremely convenient. Dialysis is however, much more cost effective and so if I am not in a hurry for a sample I tend to use it. Further, dialysis does not rely on concentration of a sample and so may be more ‘gentle’ for your sample. The Spectra/Por® range of tubing definitely proves reliable, cost effective and widely applicable.

Review Synopsis

• **Product**
  Spectra/Por® 1, 2, 3, 4, 5, 6, and 7 Dialysis Tubing by Spectrum® Laboratories Inc.

• **The Good**
  Extensive range of tubing to cover all your dialysis needs. Almost ready to use. MWCOs are typically reliable. Cost effective.

• **The Bad**
  Contamination from sulphur or heavy metals (except for Spectra/Por® 7) maybe an issue, although this is accounted for with cleaning solutions and tubing that has low levels of contamination.

• **The Bottom Line**
  It is just dialysis tubing but it is reliable, comes in a wide range of MWCOs (making it widely applicable) and it is a simple technique.
Calbiochem®

Introducing InhibitorSelect™ Protein Kinase Library I

Library I contains 80 well characterised, potent protein kinase inhibitors in a convenient 96 well plate in 50 µl aliquots supplied at a concentration of 10 mM in DMSO.

Ideal for:
- Target identification in drug discovery
  Screen for small molecule inhibitors that block receptor mediated signal transduction
- Biochemical pathway analysis
  Screen small molecule inhibitors that may affect either upstream or downstream targets
- Screening of new protein kinases
  Profile against inhibitors with known biological activity and structure
- Other pharmaceutical applications
- Useful for cell phenotypic screening
- Cell-based assays

Targets:

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Product Cat. No.
InhibitorSelect™ Protein Kinase Library I 539744-1
Advantages of InhibitorSelect™ Protein Kinase Library I

Format
- Convenient, reusable 96 well, polypropylene, non-pyrogenic black V-bottom microplate
- Corning™ 96 well robolid microplate seal provides individual silicone seal for each well of plate with resiliency for DMSO
  > Protects DMSO solutions from moisture
  > Avoids cross contamination
  > Can be applied and removed a number of times

Inhibitors
- Cell-permeable for use in cell based-assays
- ATP-competitive
- Stable in DMSO
- 50 µl/well
- Structurally diverse selection
- Multiple inhibitors against selected targets
- Potent, with well-established biological activities
- Individual inhibitors are available in larger quantities

Comprehensive Documentation
- Structure Data Files (SDF)
- Published IC<sub>50</sub> values
- CAS numbers
- Literature citations
- PubChem compound ID (where available)
- Inhibitor description
- Molecular weight

Highest Quality Control
- Purity of every inhibitor solution is tested by HPLC (≥ 95%)
- Lot specific data for every inhibitor in solution is provided

For more information contact your local VWR sales office.
Thermo Scientific HeavyPeptides™
A major step towards an effective detection of doping

Thermo Scientific HeavyPeptides™ are used for an easy and readily reproducible method for the absolute quantification of two biomarkers for growth hormone (GH) abuse.

A recent issue of the Journal of Chromatography includes a scientific paper recommending HeavyPeptides™ from the Life Science Research Custom Biopolymers unit in Ulm, Germany, in the identification of growth-hormone abuse by athletes. HeavyPeptides are custom synthetic peptides modified with heavy amino acids (15N, 13C).

The paper — titled Development of an Absolute Quantification Method Targeting Growth Hormone Biomarkers Using Liquid Chromatography Coupled to Isotope Dilution Mass Spectrometry — was written by mass-spectrometry specialists at the University of Liege, Belgium (and from Germany). They include Stephanie Kirsch, Joelle Widart, Jean-Francois Focant and Professor Edwin De Pauw. Also contributing to the paper was Joel Louette, Custom Biopolymers commercial director and biochemist, who helped develop the product. The authors detail their work with a new technique for absolute quantification of proteins. This study describes an approach to quantify two specific proteins (IGF-1 and IGFBP-3), which are biomarkers for the abuse of growth hormones (illicit doping in sports). The method is based on isotopic dilution with stable isotopic labeled peptide standards (HeavyPeptides™ AQUA).

The detection of growth hormone (GH) abuse is difficult, because it is also endogenously (naturally) produced and not stable, which prevents the detection from the day after the abuse. The two proteins examined are produced notably under

GH-control and are known as suitable biomarkers for it. The absolute quantification of these markers in a given sample allows the researcher to identify abnormal yields and suggest an abuse of GH. Commonly used methods (like ELISA, DIGE, SILAC) suffer from several difficulties like false-positive-results. The authors present a method allowing the simultaneous absolute quantification of IGF-1 and IGFBP-3, the two biomarkers of growth hormone abuse.

In this method proteins are quantified using Thermo Scientific HeavyPeptides™ AQUA Kits (AQUA stands for absolute quantification) and analysed with mass spectrometry.

**Advantages**

- High precision, absolute quantification
- Higher accuracy, sensitivity and specificity
- Easy to order custom-made HeavyPeptides™
- Quantification of low abundance proteins
- Economically viable for high throughput screening

**Applications**

- Biomarker verification and validation
- Pharmacokinetics
- Metabolomics
- Clinical biochemistry (drug and metabolite monitoring)
- Anti-doping testing
- Protein expression
- Confirmation of RNAi experiments
- Cell signalling profiling
- Allergen quantification

### Available Amino acids:

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For more information on the full range of HeavyPeptide™ Aqua kits contact your local VWRbioSciences specialist.
PerfectPro System – expression, purification, detection and assay of His-tagged proteins

5 PRIME’s pp-Vector family – efficient cloning and expression of His-tagged proteins

5 PRIME’s pPP-Vector Family allows efficient cloning and expression of His-tagged proteins. You can make your choice from a variety of vectors to express proteins with an N-terminal or C-terminal His-tag.

5 PRIME pPP-Vector family provide
- Highly efficient expression – High protein yields
- Multiple cloning options – His-tag at the N- or C-terminus
- Vectors for versatile use – e.g.: PCR cloning, expression in non bacterial systems

High protein yields through highly efficient expression
PerfectPro vectors are designed for cloning a gene of interest via restriction enzyme(s) and ligase and subsequent high-level expression from the powerful phage T5 promoter.

All pPP vectors include: Optimised promoter/operator elements, a synthetic ribosomal binding site RBSII for efficient translation, the β-lactamase gene for selection with ampicillin, the 6-His-tag coding sequence in 5’ or 3’ position for flexible expression, a large multiple cloning site and translational stop codons in all reading frames for convenient preparation of the expression constructs.

Highly efficient Ni-NTA purification under native and denaturing conditions

The Ni-NTA purification uses the affinity of neighbouring histidines of the His-tag sequence and an immobilised Ni\textsuperscript{2+} ion. The metal is held by chelation with reactive groups covalently attached to a solid support. The most efficient chelator is nitriloacetic acid (NTA), which has four available interaction sites with the metal ion. The strong chelator minimises the leaching of metal during purification and is compatible with stringent wash conditions.

The PerfectPro Purification System is a:
- One-step purification – From the crude lysate to > 95% pure protein
- Powerful system – High binding capacity using Ni-NTA
- Outstanding purity – Stringent washing and no metal leaching
- Versatile use – Native and denaturing conditions

The Ni-NTA Matrices of the PerfectPro System are PerfectPro Ni-NTA Agarose for efficient purification by gravity-flow chromatography or PerfectPro Ni-NTA Superflow for use in FPLC systems. Both matrices demonstrate a high binding capacity of 5-20 mg/ml with allows purification at high yields with over 95% homogeneity of the recombinant proteins in one-step. Due to the resistance against various chemicals purification in native or denaturing conditions with a large number of purification protocols are possible.

PerfectPro is a complete and integrated system for expression, purification, detection and assay of His-tagged proteins. For all steps 5 PRIME provides easy to use and efficient tools.
Sensitive detection with Anti His Antibodies and Anti His HRP Conjugates

The PerfectPro Anti-His Antibodies and HRP Conjugates provide:
- Specific detection – High sensitivity detection of His-tag protein
- Versatile use – Detection of N-terminal, C-terminal, and internal His-tag
- Fast procedure – Direct detection of Anti-His HRP Conjugates
- Flexible applications – Colorimetric, chemiluminescent and fluorescent assays

5 PRIME’s PerfectPro System is a comprehensive set of tools for expression, purification, detection and assay of His-tagged proteins.

The mouse monoclonal IgG1 antibodies that have high affinity and specificity for the 6xHis tag and show only a negligible cross-reactivity with crude E. coli, yeast, mammalian, or insect cell lysates.

RGS·His Antibody recognises the RGS(His)4 epitope whereas Penta·His Antibody and Tetra·His Antibody can bind to 6xHis-tagged proteins expressed from any vector and do not require any additional amino acids. Regardless of the surrounding amino-acid context, Penta-His Antibody recognises five consecutive histidine residues, and Tetra-His Antibody recognises four, allowing them to bind to partially hidden 6xHis tags that other anti-His antibodies do not recognise. For making the right choice of antibody for your application the Anti-His Antibody Selector Kit is a cost-efficient solution to compare and determine the optimal antibody.

Optimised assays for His-tagged proteins with PerfectPro Ni-NTA MagBeads, Ni-NTA HisPrime Strips and Plates

The Ni-NTA HisPrime Strips and Plates provide:
- Optimal presentation of assay partners – Direct immobilisation
- Highly standardised and reproducible conditions – Universal chemistry
- Minimised non-specific binding – High affinity and enhanced signal-to-noise ratio

Optimal presentation through direct immobilisation of assay partners

Ni-NTA HisPrime Strips and Plates provide Ni-NTA in a solid-phase, multiwell format for 6xHis tag assay of proteins or other molecules. Tagged molecules are not denatured and are bound to the strips in a uniform orientation. They are conformationally active while remaining available to detection reagents (antibodies or any interacting molecule).

Ordering information, see next page
## PerfectPro System
### Ordering information

<table>
<thead>
<tr>
<th>Expression</th>
<th>PerfectPro Kit</th>
<th>PerfectPro vectors (pPP)</th>
<th>Cat. No.</th>
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### Protein detection

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<td>RGS Anti-His Antibody BSA-free (100 µg)</td>
<td>RGSHis4</td>
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<td>Tetra-His Antibody BSA-free (100 µg)</td>
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<td>Penta-His Antibody (100 µg)</td>
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<td>733-1229</td>
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<tr>
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### Protein assays

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