

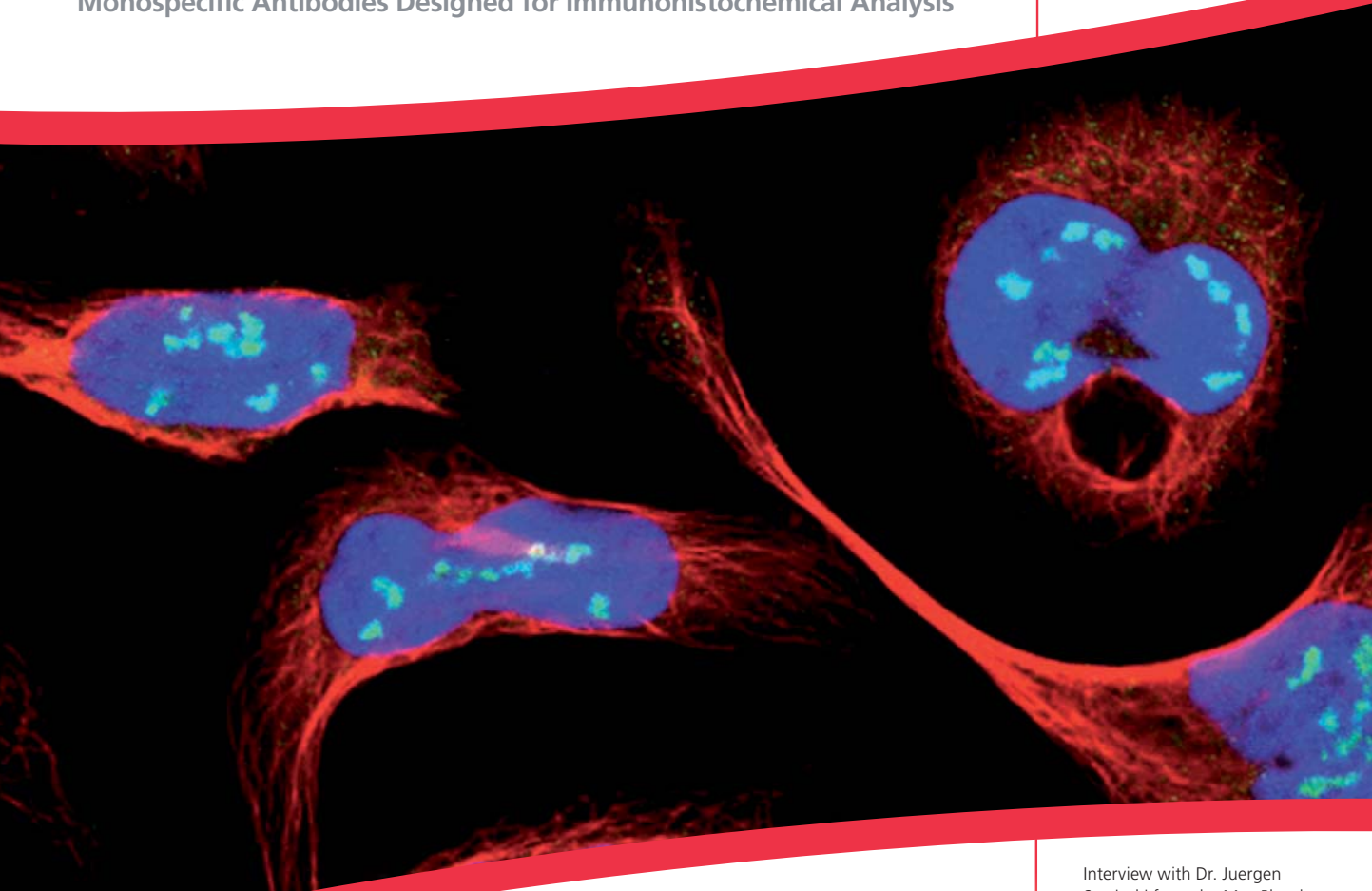
BioFiles

INVITRO

Issue 4 • 2009



Sigma's new range of Prestige Antibodies®
Monospecific Antibodies Designed for Immunohistochemical Analysis



Interview with Dr. Juergen Sawinski from the Max Planck Institute for Biological Cybernetics in Tuebingen

Staining Protocol Series:
Modified Silver Stain
by GMS and Steiner

Increase Bioanalytical Assay
Speed via Phospholipid Removal
Using HybridSPE™-Precipitation
Technology

Bacillus: A Ubiquitous Bacteria
Genus

Centrifugation Media from Sigma-Aldrich



Centrifugation Media

Histopaque®-1077

1.077 g/mL at 25 °C, aseptically filled, for *in-vitro* diagnostic use (Mat. No. 10771-500ML; 10771-6 x 100ML)

ACCUSPIN System-Histopaque®-1077

Box of 100 tubes. Each 50 ml tube contains 15 ml Histopaque 1077-1 and will separate between 15 to 30 ml of anticoagulated blood (Mat. No. A0561-100 x 15ML)

Percoll®

pH 8.9±0,3 (20 °C), aseptically filled (Mat. No. P1644-500ML; P1644-1L)

Ficoll® solution

Type 400, 20 % in H₂O, 0.2 µm filtered (Mat. No. F5415-25ML; F5415-50ML)

Cesium chloride

SigmaUltra, ≥99.5 % (titration) (Mat. No. C3309-50G; C3309-250G)

Sucrose

for molecular biology, ≥99.5 % (GC) (Mat. No. S0389-500G; S0389-1KG)

33 %
Discount

- A complete line of products for the separation or extraction of leukocytes, viruses, DNA, RNA, and more
- Special line of products for "*in-vitro* diagnostic use" in clinical laboratories
- Histopaque® system for easy separation of lymphocytes from whole blood

To order, please contact your local sales office and quote Promo Code: **X65**

Web ordering: sigma-aldrich.com/euvoucher

Promotion is valid until June 30th 2009.



Our Innovation, Your Research – Shaping the Future of Life Science

Dear Colleague,



Sigma-Aldrich Chemie GmbH
Walter Gmelin, PhD

Sigma is a trusted partner for Antibodies and Immunohistochemistry in the field of Medical Research. We have significantly enhanced our antibody offering including the exciting new range of Prestige Antibodies®.

Prestige Antibodies® powered by Atlas Antibodies AB are accompanied by over 500 immunohistochemical images. While most commercially available antibodies on the market are validated solely by the supplier, Prestige Antibodies – powered by Atlas Antibodies – are supported by the publicly available data of the Human Protein Atlas (HPA), a part of HUPO's Human Antibody Initiative.

In this issue, Marianne Hansson gives a comprehensive overview about the application range and the development of Prestige Antibodies from the Human Protein Atlas programme. The HPA programme was initiated at the Royal Institute of Technology in Uppsala, Sweden. It generates protein expression profiles of the non-redundant set of human proteins, presented as immunohistological images from the majority of human tissues. All images are annotated and made publicly available via an open access database, the Human Protein Atlas (proteinatlas.org). An ambitious and rigorous quality-control process has been developed by which all antibodies have to pass a set of criteria prior to being tested by immunohistochemistry (IHC) and other methods.

We are also proud to present an interview with Juergen Sawinski, a postdoctoral fellow at the Max Planck Institute for Biological Cybernetics. He tells us how he started his scientific career as a physicist, yet finds himself conducting biomedical research. A story about how multiple disciplines work together and why we love being in research.

In addition, you will find an article describing how to increase your bioanalytical assay speed via phospholipid removal using HybridSPE™-Precipitation technology, plus 2 new protocols from our Stain Protocol series.

We hope you enjoy reading this informative issue.

Kind regards,

Walter Gmelin, PhD
Product Manager Life Science Europe

Table of Contents

Interview with Juergen Sawinski	4
Prestige Antibodies®	7
Silver Stain (modified GMS)	15
Silver Stain (modified Steiner)	16
Increase Bioanalytical Assay Speed via Phospholipid Removal using HybridSPE™-Precipitation Technology	18
<i>Bacillus</i> : A Ubiquitous Bacteria Genus	22

Cover illustration: Confocal Images of immunofluorescent staining of the human cell line. The third channel is turned on for a three-colour overlay view of cytoskeleton (red), nuclei (blue) and the anti HMBG2 Prestige Antibody (green).

Interview with Juergen Sawinski

Post-doctoral fellow at the Max Planck Institute Tuebingen in Germany



CURRICULUM VITAE



Education

- 10/1993–07/1996** **University of Ulm**
Degree: intermediate diploma in physics
- 10/1996–05/2002** **University of Heidelberg**
advanced study period
Degree: graduate physicist
- 01/2003–12/2005** University of Heidelberg, physics doctorate accomplished at the
Max Planck Institute for Medical Research
Topic: "Development of a head-mount fibre scanning system for imaging in vivo"
Degree: Doctor of Science

Experience

- 03/1994–08/1997** **AIESEC** Trainee care, marketing, responsible for several projects
- 07/1997–10/1997** **Max Planck Institute for Particle Physics,** Heidelberg, group of Prof. Dr. B. Povh, research project: simulation of particle detectors
- since 1998** **LaTeX**-specialist for several books ("**Teilchen und Kerne**", B. Povh, K. Rith, C. Scholz and F. Zetsche, Springer Publishing New York, Berlin, Heidelberg, Tokyo, among others)
- 01/2006-10/2007** Postdoc at Max Planck Institute for Medical Research, Biomedical Optics (**Prof. Dr. Winfried Denk**)
- since 10/2007** Postdoc at **Max Planck Institute for Biological Cybernetics,** Tuebingen, NWG Jason Kerr

Publications

- J. Sawinski, **Development of a head-mount fibre scanning system for imaging in vivo**, Heidelberg (Ruprecht-Karls-Universität Heidelberg), (2005)
- Peixin Zhu, et al., **Silencing and Un-silencing of Tetracycline-Controlled Genes in Neurons**, PLoS ONE, (2007), 2(6): e533
- J. Sawinski, W. Denk, **A miniature random-access fibre scanner for in vivo multi-photon imaging**, J. Appl. Phys. 102, 034701 (2007)

Dr. Juergen Sawinski is conducting post-doctoral research in the laboratory of Dr. Jason Kerr, as part of a research group at the Max Planck Institute for Biological Cybernetics. Juergen studied physics, but moved from his first interest for elementary particle physics to biophysics.

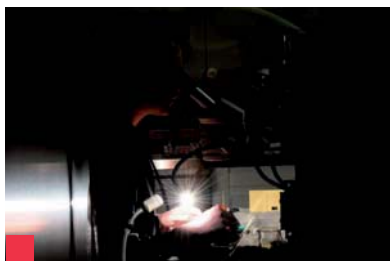
IN VITRO: Juergen, you are a physicist working at the forefront of medical research. We are curious to know how you found yourself in this field of research.

JS: You are right, it wasn't my initial plan to do research in neurobiology. My interest started with an exciting discussion one evening with one of my roommates over several glasses of wine.

At first, I had no idea about the topic, but very soon became captivated. My friend opened me up to the opportunities you have as a physicist working in a multidisciplinary environment. That led me to start my scientific career at the Max Planck Institute for Medical Research as a diploma student in the department for biomedical optics.

IN VITRO: Biomedical optics sounds very technical. What was the scope of your research?

JS: The department goal was the development and application of new optical methods for biomedical research. Biological function often occurs in a complex tissue environment and the challenge was to conduct physical measurements in that matrix. For this type of investigation the department uses optical microscopy to study living tissue with high spatial resolution. In my diploma thesis we were able to show that this type of investigation can be applied to the living tissue of a rat's brain.



IN VITRO: Optical microscopy doesn't sound like a new way of investigating tissues.

JS: The set-up of the microscope is in fact very basic. You can compare the simple optics with the first microscopes being developed at the beginning of the 17th century! But we had good reasons for this experimental set-up. Our next goal was to miniaturise the microscope, and the simpler the optics, the easier it is to reduce the size.

The technique we use is 2-photon microscopy. Multi-photon microscopy has now become an established technique to identify specific regions in biological specimen. In our group we apply that technique to investigate neuro-physiological signal transmission in living complex tissues like our current studies on brains of freely moving rats.

IN VITRO: Can you explain how you can observe signals in brains of freely moving animals?

JS: The microscope is so small that we can easily attach it to a hat worn by the rat while the animal is moving around. We can define the active parts of the rat brain by time-resolved fluorescence measurements. At the beginning we could only do that in two

dimensions, but we are currently evolving solutions to define the regions in three dimensions, too.

This will help us to understand far better how signal transduction works in a complex system like our brain.

IN VITRO: It sounds as though your research went quite smoothly. Is that true?

JS: No, not at all, I believe that most of us have these frustrating moments where nothing seems to develop and you have a tough time motivating yourself.

In my case a diploma student built an operating system of our laser with all the different optical mirrors being perfectly arranged. Unfortunately we had to move the complete laser construction after which the set-up didn't work any more. After several attempts to get it working, I started to rebuild the complete system from scratch. At one point my tutor came in and asked his standard question, whether the system was now running. I said to him quite frankly that I had tried everything, but if didn't work now I had no idea what else to do. He took a look at the set-up, touched one of the mirrors – and the laser started to work.

It was one of those moments where you love and hate research at the same time!

IN VITRO: What will be next – where do you see your future?

JS: I have learned from experience that you can't exactly plan your future. More than 10 years ago I wouldn't have imagined working in medical research as a physicist. The opportunity came up and I am happy working in such an environment with an interdisciplinary group of scientists and excellent capabilities. Let's see where and how that takes me.



Prestige Antibodies®



Powered by  **ATLAS**
ANTIBODIES



Turn your research into a masterpiece with Prestige Antibodies®

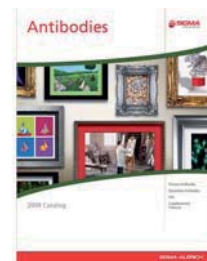
The most highly characterized antibodies in the industry — powered by Atlas Antibodies.

- Over 3,800 antibodies, covering 3,600 protein targets
- Validated by the Human Proteome Resource (HPR)
- Standardized in universal protocols
- Over 500 IF, IHC and WB images per antibody
- All data conveniently searchable online

Visit sigma.com/prestige for more information.

Our Innovation, Your Research — Shaping the Future of Life Science

Prestige Antibodies is a registered trademark of Sigma-Aldrich Biotechnology L.P. and Sigma-Aldrich Co.



**Now available,
the new Antibodies
Catalog 2009–2010!**



Our Innovation, Your Research – Shaping the Future of Life Science

Prestige Antibodies®

Monospecific Antibodies Designed for Immunohistochemical Analysis



Sara A. Gunnerås, Charlotta Agaton, Soraya Djerbi and Marianne Hansson
Atlas Antibodies AB, AlbaNova University Centre, SE-106 91 Stockholm, Sweden

Introduction

Well-characterised antibodies are essential tools for protein studies and global proteomics analysis, as well as for clinical diagnostics. Although the production of antibodies is a well-established process, and a large number of antibodies are commercially available through many vendors, specific antibodies still do not exist for the majority of human proteins. An underlying factor limiting the available antibody repertoire is that commercial production tends to focus on popular targets. The current needs of the proteomics community demand a far more global approach.^{1,2} A second significant issue is the lack of a universally defined standard for antibody quality. This makes it difficult to compare antibodies of various sources without committing resources to using the antibody in its final application. Initial standardised testing for specificity and sensitivity followed by a thorough characterisation would be of great interest to the end user, but this is a costly endeavour and most efficiently accomplished on a larger scale.

At present, there are only a handful of large-scale high-throughput antibody production efforts initiated around the world.³ One such initiative is the Swedish Human Protein Atlas (HPA) programme.⁴ The aim of this programme is to explore the entire human proteome using an antibody-based proteomics approach.^{5,6} Specifically, the HPA programme generates protein expression profiles of the non-redundant set of human proteins, presented as immunohistological images from the majority of human tissues. All images are annotated and made publicly available via an open access database, the Human Protein Atlas (proteinatlas.org). An ambitious and thorough quality-control process has been developed by which all antibodies have to pass a set of criteria prior to being tested by immunohistochemistry (IHC) and other methods.⁷

The Human Protein Atlas Programme

The Swedish Human Protein Atlas (HPA) programme is an academic initiative, headed by Professor Mathias Uhlén at the Royal Institute of Technology in Stockholm, Sweden and the Rudbeck Laboratory in Uppsala, Sweden. The vision of the HPA programme is to systematically generate quality-assured antibodies to all non-redundant human proteins, and to use these reagents to functionally explore human proteins, protein variants and protein interactions. At present, 50 new antibodies are generated per week along with 50,000 new IHC images. In order to manage the large amount of material and data generated, methodologies have been developed to support high-throughput systems including data collection, image handling and storage.

Antibody Development and Quality Control

The HPA proteomics approach is based on affinity-purified polyclonal antibodies (mono-specific antibodies, msAbs) raised towards bioinformatically designed Protein Epitope Signature Tag (PrEST) antigens. The PrEST antigens, the mono-specific antibodies and the resultant images for the Human Protein Atlas are generated in a high-throughput manner as outlined in **Figure 1**.

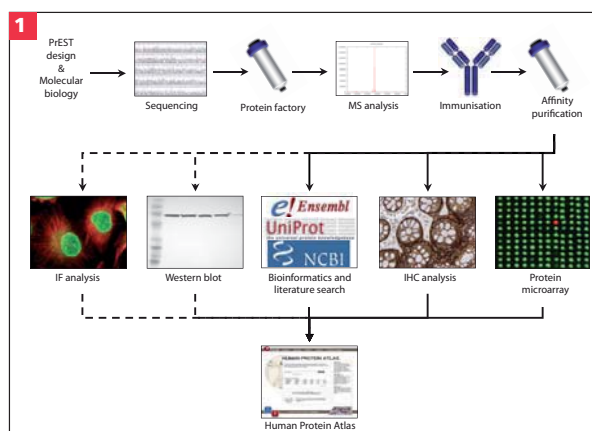


Figure 1: Schematic overview of the workflow and control steps in the HPA antibody development process (top row) and the various expression profiling analyses and validation steps (bottom row)

The initial step of the process is the antigen design. Tailor-made bioinformatics software based on the Basic Local Alignment Search Tool (BLAST) function is used to design the PrEST antigens. The scanning procedure permits the selection of fragments of a specified size, between 50–150 amino acids, with a minimal sequence similarity to other human proteins. Further, this programme allows for the avoidance of certain restriction enzyme sites, transmembrane regions, and signal peptides.

The computer selected PrEST regions are RT-PCR amplified from pools of human total RNA and cloned into an expression vector as a fusion to a histidine tag and an albumin-binding protein (ABP).⁸ All recombinant PrEST clones are fully sequenced to verify the

correct insert and that no polymerase-introduced mutations are present. The PrEST sequence analysis is the first of several quality control steps that must be passed before further processing (**Figure 1**).

The sequence-verified PrEST clones are expressed in *E. coli* and the produced PrEST antigens are affinity purified by immobilised metal ion chromatography under denaturing conditions. The purified PrEST antigens are quality controlled by mass spectrometry (MS) for sequence accuracy, by SDS-PAGE analysis for protein purity analysis, and by bicinchoninic acid assay (BCA) for protein concentration determination.

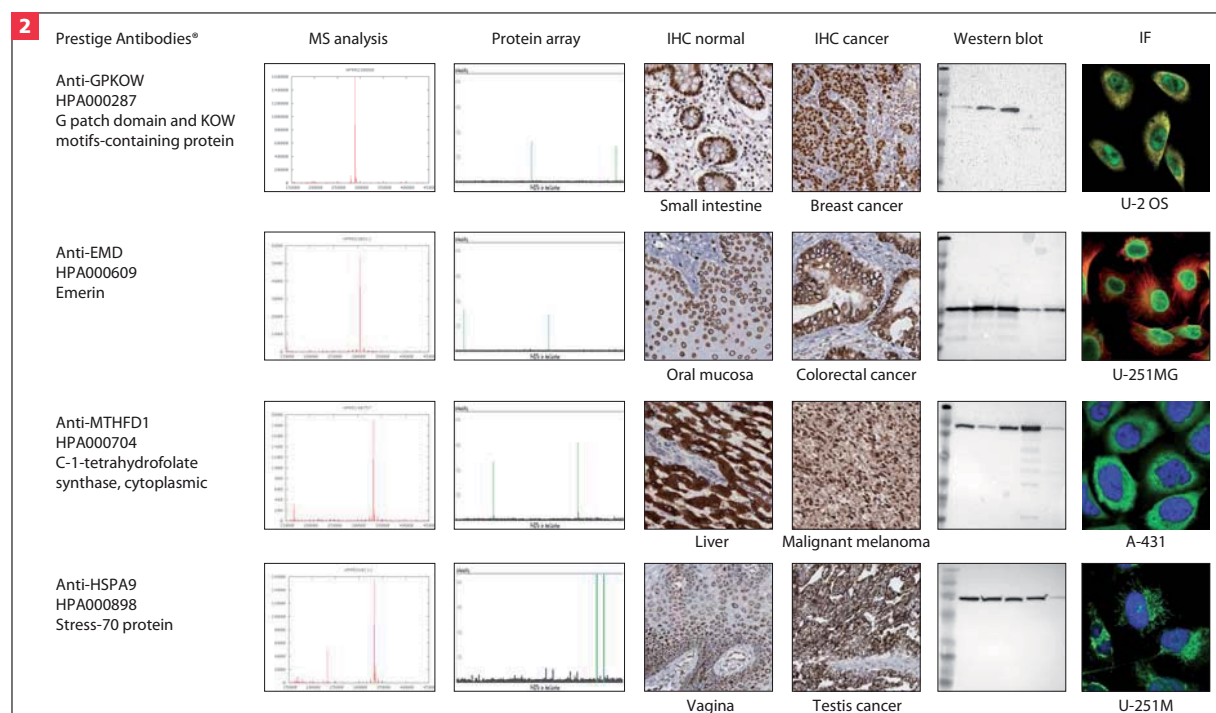


Figure 2: Four Examples of Prestige Antibodies with Corresponding Quality Control Data and Expression Profiling Results

MS analysis. Mass spectrometry analysis of the PrEST antigens are presented in the first lane (MS analysis). A single peak of correct mass verifies production and purification of the correct PrEST antigen.

Protein array. Binding specificity for each purified Prestige Antibody is verified by protein microarray analysis (lane 2, Protein array). In the initial phase of the HPA programme, each PrEST antigen was spotted in duplicate and two peaks verify antigen-specific binding as shown for each of the four Prestige Antibodies in **Figure 2**. However, at present 384 different PrEST antigens have been spotted, each as a single spot, allowing analysis of background reactivity towards a larger set of PrEST antigens. Depending on PrEST array set-up, the resulting images on the Human Protein Atlas will show two specific peaks (duplicate spots) or one specific peak (single spot).

IHC normal tissue. In lane 3 (IHC normal), one out of 144 images from IHC-stained normal tissue samples are shown. The tissue localisation of a protein is shown by specific binding of an antibody to its corresponding antigen in IHC, indicated by brown staining. The tissue section is counterstained with hematoxylin to enable visualisation of microscopical features. Hematoxylin stains both cells and extracellular material in blue.

IHC cancer tissue. IHC expression analysis of cancer tissue samples is performed the same way as IHC staining of normal tissue, with the binding of the antibody to its antigen resulting in a brown staining pattern. One out of 432 images from IHC-stained cancer tissue samples is shown in lane 4.

Western blot. The Prestige Antibodies are analysed by Western blot against total protein lysates from two human cell lines (RT-4 and U-251MG), two human tissues (liver and tonsil) and human plasma in a high-throughput standardised manner (lane 5, Western blot).

Immunofluorescence (IF). A large number of Prestige Antibodies have been used for subcellular localisation by immunofluorescence (lane 6, IF). The protein targeted by the Prestige Antibody is shown in green, the endoplasmic reticulum in yellow, cytoskeleton in red and nuclei in blue.

The purified PrEST proteins serve various purposes in the downstream antibody development and control process, first as antigens for immunisations, secondly as PrEST-ligands coupled to affinity columns for antibody purification, and thirdly as ligands on protein arrays used to help ensure specific antibody binding to the complementary antigen.

Mono-specific polyclonal antibodies are purified from raw antiserum in a three-step process, starting with depletion of unwanted specificities, i.e. His6-ABP affinity tag-specific antibodies. The flow-through is then passed through a PrEST antigen column, allowing capture of anti-PrEST specific antibodies. Finally, after washing, captured antibodies are eluted and loaded onto a desalting column for buffer exchange. The purified mono-specific polyclonal antibodies are then analysed for specificity on a protein array consisting of 384 different PrEST antigens spotted on glass slides, including the matching PrEST antigen to the mAb to be tested.⁹ An example of a typical PrEST array analysis is shown in **Figure 2**. It illustrates specific reactivity between the mAb and the corresponding PrEST protein, which is contrasted against low background reactivity to other PrEST proteins.

Human Protein Expression Profiling

All mono-specific antibodies that pass the protein array control step are tested on a standard set of human Tissue Microarrays (TMAs), carefully selected to be tissue representative. The final set of TMAs stained for full protein expression profiling includes formalin-fixed paraffin-embedded samples from 48 non-neoplastic, morphologically normal human tissues in triplicate and tissues from 20 common cancer types derived from 4–12 individuals performed in duplicate. In addition, each antibody is used to stain Cell Microarrays (CMAs) from 47 cell lines and 12 samples of primary blood cells, each performed in duplicate.¹⁰

In total, more than 700 high-resolution IHC images, each representing a tissue or cell section, are generated per antibody, and uploaded onto the Human Protein Atlas. With the aid of a web-based annotation programme, certified pathologists or specially trained personnel annotate all newly tested antibodies. One or several representative cell types are annotated for each tissue and given a staining score. To ensure high quality, all annotated tissue images are curated by a second person. Annotation of cell sample images is done automatically by image analysis software.¹¹

Each antibody in the Human Protein Atlas is given a validation score. A validation score is assigned based on (i) the IHC staining pattern, (ii) available bioinformatic data, and (iii) whether supporting data exists in the literature. The validation score indicates how well the quality assurance data supports the specificity of the antibody against the expected human target protein.

In order to obtain a *high* validation score, two independent antibodies targeting the same protein and showing similar staining patterns are required. In addition, the staining pattern must be consistent with experimental and/or bioinformatic data.

A *medium* score is assigned when the staining pattern is consistent with experimental and/or bioinformatic data. When the staining pattern is only partially consistent with experimental and/or bioinformatic data, a low score is assigned. For profiling of human proteins where experimental and/or bioinformatic data is not available, or the staining pattern is not consistent with such data, the antibody is assigned a *very low* score.

All results generated, including the PrEST array result, IHC images, annotation results, literature summary and validation score, are published by the Human Protein Atlas (proteinatlas.org).

Additional Applications

All new antibodies are analysed by Western blot in a high-throughput standardised manner. Total protein lysates from two human cell lines (RT-4 and U-251MG), two human tissues (liver and tonsil) and human plasma are used to evaluate the antibody target binding in a Western blot setting. The Western blot analysis for each antibody is performed using identical set-ups with no optimisation, i.e. the same five protein lysates, as well as the same conditions are used for all antibodies. As the protein samples and running conditions are not specifically selected and adapted for each antibody and its corresponding protein, not every antibody has been successfully validated for Western blot application. However, the Western blot data do have an impact on the validation score.

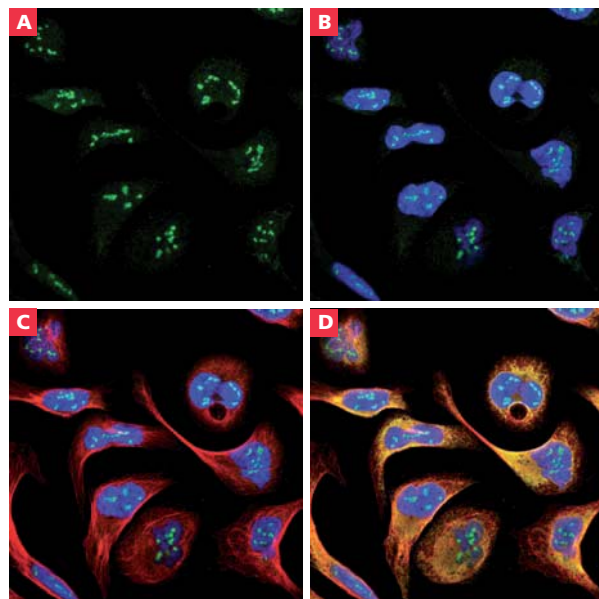


Figure 3: Confocal Images of immunofluorescent staining of the human cell line U-2OS using the nucleoli specific Prestige Antibody anti-HMBG2 (HPA0003506) in combination with organelle probes specific for the endoplasmic reticulum, cytoskeleton and nuclei. The four-colour image is acquired in four separate channels that can be viewed separately or in different combinations

Image A: View of one channel showing the Prestige Antibody staining of nucleoli in green.

Image B: A second channel is turned on for an overlay view of the control staining of nuclei (blue) and the anti-HMBG2 Prestige Antibody (green).

Image C: A third channel is turned on for a three-colour overlay view of cytoskeleton (red), nuclei (blue) and the anti-HMBG2 Prestige Antibody (green).

Image D: The fourth channel is turned on for a four-colour overlay view of endoplasmic reticulum (yellow), cytoskeleton (red), nuclei (blue) and the anti-HMBG2 Prestige Antibody (green).

In addition to the standard immunohistochemical expression profiling performed with each antibody, subcellular localisation studies by confocal microscopy and immunofluorescence (IF) staining is performed for a large number of the Prestige Antibodies[®],¹² as shown in **Figure 3**. In the Human Protein Atlas, the cell lines used for IF staining can be visualised in several ways. The protein targeted by the Prestige Antibody is shown in green, nuclei in blue, cytoskeleton in red and endoplasmic reticulum in yellow. In order to optimise the view, one or several channels can be removed and/or combined.

How to Use the Human Protein Atlas

The Human Protein Atlas currently consists of a collection of over 5.7 million images covering the majority of normal and cancer tissues as well as a large selection of cell lines and primary cells. In the current version (4.1) more than 6,100 antibodies have been screened for protein profiling of more than 5,000 human proteins. More than half of these antibodies are Prestige Antibodies targeting approximately 3,600 human proteins. New data and new features are released on an annual basis.

There are three ways of searching the database: (i) by viewing the proteins by chromosome, (ii) by using the simple search box, or (iii) by advanced search. The simple search box allows searches by gene name, gene description, antibody ID (product number) or Ensembl ID.

The advanced search tool is based on protein expression levels in all the included normal and cancer tissues, and a combination of searching criteria can be utilised, such as “and” or “and not” queries. In addition, it is possible to search for proteins differentially expressed within a tumour type, i.e. among different patients having the same cancer type. For example, the number of patients of a given tumour type that should show a specified staining intensity, higher or lower intensity as compared to the rest of the patients of the same tumour type, can be selected. For a detailed description of the advanced search option and *in silico* biomarker discovery, refer to Björling and co-workers.¹³

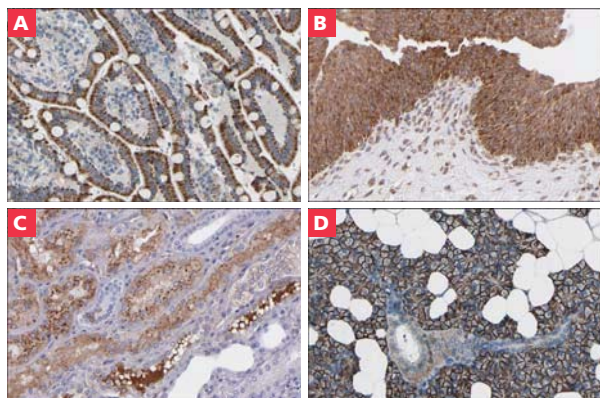
A search query results in a list of proteins/antibodies fitting the search criteria. The expression profile overview page is viewed by clicking on the antibody ID. The normal and cancer tissues can be sorted in alphabetic or histogenic order. All tissues and cells are given a protein expression level score, visualised by a coloured pie chart, ranging from strong staining (red) to no staining (white). The pie chart system gives a quick overview of the protein expression profile. An annotation summary provides a brief description of the major findings from the IHC analysis. The high-resolution IHC images are viewed by clicking on the tissue type name. To see specific detail, all images can be viewed at higher magnification.

Conclusions

The lack of high-quality protein-affinity reagents for extensive and efficient proteomics studies is a widely accepted problem.¹ Here we have described one effort to increase the availability of anti-human protein antibodies, as well as the use of antibody-based proteomics to explore the human proteome. With a proven high-throughput strategy for development of well-characterised antibodies against human protein targets, and the subsequent use of these antibodies in an extensive protein expression profiling study, the Human Protein Atlas (HPA) programme will have a major impact on proteomics research for many years to come.

The current HPA antibody development pipeline generates about 2,000 new well-characterised anti-human antibodies every year. These Prestige Antibodies® are valuable tools in protein research and proteomics studies, not only for the protein expression studies performed by the HPA programme, but also for other scientific researchers around the world. The main application for which all Prestige Antibodies are validated is immunohistochemical staining of paraffin-embedded, formalin-fixed tissue and cell samples. In addition, a large number of the Prestige Antibodies have been successfully used in Western blot analyses and immunofluorescence staining.

With the overall objective of achieving the first draft of a Human Protein Atlas covering the majority of the non-redundant human proteins by the year 2014, the HPA initiative is well positioned to substantially increase the number of available anti-human protein-affinity reagents of exceptional quality and thus revolutionise how proteomic research is conducted.



References

- 1] Blow, N. 2007. Antibodies: The generation game. *Nature* 447(7145): 741–744.
- 2] Editorial: Proteomics' framework for success? 2007. *Nature Methods* 4(12): 1–2.
- 3] Persson, A. *et al.* 2006. A human protein atlas based on antibody proteomics. *Curr. Opin. Mol. Ther.* 8(3): 185–90.
- 4] Uhlén, M.E. *et al.* 2005. A human protein atlas for normal and cancer tissues based on antibody proteomics. *Mol. Cell. Proteomics* 4(12): 1920–32.
- 5] Kampf, C. *et al.* 2004. Antibody-based tissue profiling as a tool in clinical proteomics. *Clin. Proteomics* 1(3–4): 285–300.
- 6] Uhlén, M. *et al.* 2005. Antibody-based proteomics for human tissue profiling. *Mol. Cell. Proteomics* 4(4): 384–393.
- 7] Hober, S. *et al.* 2007. Human protein atlas and the use of microarray technologies. *Curr. Opin. Biotech.* 19: 1–6.
- 8] Agaton, C.J. *et al.* 2003. Affinity proteomics for systematic protein profiling of chromosome 21 gene products in human tissues. *Mol. Cell. Proteomics* 2(6): 405–414.
- 9] Nilsson, P.L. *et al.* 2005. Towards a Human Protein Atlas: mono-specific antibodies for tissue profiling. *Proteomics* 5(17): 4327–4337.
- 10] Andersson, A.C. *et al.* 2006. Analysis of protein expression in cell microarrays: a tool for antibody-based proteomics. *J. Histochem. Cytochem.* 54(12): 1413–1423.
- 11] Strömberg, S. *et al.* 2007. A high-throughput strategy for protein profiling in cell microarrays using automated image analysis. *Proteomics* 7(13): 2142–2150.
- 12] Barbe, L. *et al.* 2008. Towards a confocal subcellular atlas of the human proteome. *Mol. Cell. Proteomics*, 7(3): 499–508.
- 13] Björling, E. *et al.* 2007. A web-based tool for in silico biomarker discovery based on tissue-specific protein profiles in normal and cancer tissues. *Mol. Cell. Proteomics*, Oct 3 [Epub ahead of print].

As published in *BioTechniques*, biotechniques.com Vol. 44, No. 4, 2008.

Figure: Immunohistochemistry

Image A: Anti-PIK3CA: (HPA009985): Immunoperoxidase staining of formalin-fixed, paraffin-embedded human duodenal tissue showing cytoplasmic staining of glandular cells.

Image B: Anti-TNRC6B: (HPA003180): Immunoperoxidase staining of formalin-fixed, paraffin-embedded human urinary bladder tissue showing cytoplasmic and/or membranous staining of surface epithelial cells.

Image C: Anti-CD44: (HPA005785): Immunoperoxidase staining of formalin-fixed, paraffin-embedded human salivary gland tissue showing membranous staining of glandular cells.

Image D: Anti-KNG1: (HPA001616): Immunoperoxidase staining of formalin-fixed, paraffin-embedded human kidney tissue showing granular cytoplasmic and/or membranous staining of cells in tubuli as well as extracellular positivity.

We've listed only a few of the currently available 3,800 Prestige Antibodies® powered by Atlas Antibodies. For a complete list and to learn more about the most highly validated antibodies in the industry, visit sigma.com/prestige

Product Name	Host	Form	Gene Symbol	Species Reactivity	Application	Cat. no.
Anti-ALCAM	rabbit	affinity isolated antibody	ALCAM, human	human	IHC (p), PA	HPA010926-100UL
Anti-APOBEC3G	rabbit	affinity isolated antibody	APOBEC3G, human	human	IHC (p), PA, WB	HPA001812-100UL
Anti-ARG1	rabbit	affinity isolated antibody	ARG1, human	human	IHC (p), PA, WB	HPA003595-100UL
Anti-BCHE	rabbit	affinity isolated antibody	BCHE, human	human	IHC (p), PA	HPA001560-100UL
Anti-BNIP3	rabbit	affinity isolated antibody	BNIP3, human	human	IHC (p), PA	HPA003015-100UL
Anti-CA12	rabbit	affinity isolated antibody	CA12, human	human	IHC (p), PA	HPA008773-100UL
Anti-CD44	rabbit	affinity isolated antibody	CD44, human	human	IHC (p), PA, WB	HPA005785-100UL
Anti-CDH13	rabbit	affinity isolated antibody	CDH13, human	human	IHC (p), PA	HPA001380-100UL
Anti-CP	rabbit	affinity isolated antibody	CP, human	human	IHC (p), PA	HPA001834-100UL
Anti-DCN	rabbit	affinity isolated antibody	DCN, human	human	IHC (p), PA	HPA003315-100UL
Anti-DICER1	rabbit	affinity isolated antibody	DICER1, human	human	IHC (p), PA	HPA000694-100UL
Anti-GLA	rabbit	affinity isolated antibody	GLA, human	human	IHC (p), PA, WB	HPA000966-100UL
Anti-GNG2	rabbit	affinity isolated antibody	GNG2, human	human	IHC (p), PA	HPA003534-100UL
Anti-KNG1	rabbit	affinity isolated antibody	KNG1, human	human	IHC (p), PA, WB	HPA001616-100UL
Anti-LAT2	rabbit	affinity isolated antibody	LAT2, human	human	IHC (p), PA	HPA003462-100UL
Anti-MBNL3	rabbit	affinity isolated antibody	MBNL3, human	human	IHC (p), PA	HPA001584-100UL
Anti-MMP9	rabbit	affinity isolated antibody	MMP9, human	human	IHC (p), PA	HPA001238-100UL
Anti-OSTM1	rabbit	affinity isolated antibody	OSTM1, human	human	IHC (p), PA	HPA010851-100UL
Anti-PGRMC1	rabbit	affinity isolated antibody	PGRMC1, human	human	IHC (p), PA, WB	HPA002877-100UL
Anti-PIK3CA	rabbit	affinity isolated antibody	PIK3CA, human	human	IHC (p), PA	HPA009985-100UL
Anti-PIM2	rabbit	affinity isolated antibody	PIM2, human	human	IHC (p), PA, WB	HPA000285-100UL
Anti-REST	rabbit	affinity isolated antibody	REST, human	human	IHC (p), PA	HPA006079-100UL
Anti-S100A4	rabbit	affinity isolated antibody	S100A4, human	human	IHC (p), PA	HPA007973-100UL
Anti-SFRP2	rabbit	affinity isolated antibody	SFRP2, human	human	IHC (p), PA	HPA002652-100UL
Anti-SOX9	rabbit	affinity isolated antibody	SOX9, human	human	IHC (p), PA	HPA001758-100UL
Anti-TJP1	rabbit	affinity isolated antibody	TJP1, human	human	IHC (p), PA	HPA001636-100UL
Anti-TNRC6B	rabbit	affinity isolated antibody	TNRC6B, human	human	IHC (p), PA	HPA003180-100UL
Anti-TRPV4	rabbit	affinity isolated antibody	TRPV4, human	human	IHC (p), PA	HPA007150-100UL
Anti-VCAN	rabbit	affinity isolated antibody	VCAN, human	human	IHC (p), PA	HPA004726-100UL
Anti-VIM	rabbit	affinity isolated antibody	VIM, human	human	IHC (p), PA, WB	HPA001762-100UL

Reagents for Immunohistochemistry



33%
Discount

Phosphate buffered saline, powder, pH 7.4	(Mat. No. P3813-10PAK)
Phosphate buffered saline, tablet	(Mat. No. P4417-50TAB; P4417-100TAB)
Phosphate buffered saline	(Mat. No. P3688-10PAK)
Albumin from bovine serum	(Mat. No. A9647-100G; A9647-500G)
Trypsin from porcine pancreas	(Mat. No. T7409-10G; T7409-100G)
Trypsin from porcine pancreas	(Mat. No. T7168-20TAB; T7168-50TAB)
Proteinase from <i>Bacillus licheniformis</i>	(Mat. No. P5380-100MG; P5380-250MG)
ExtrAvidin®-Peroxidase	(Mat. No. E2886-0.2ML; E2886-1ML)
ExtrAvidin®-Alkaline Phosphatase	(Mat. No. E2636-0.5ML; E2636-5 x 0.5ML)
SIGMAFAST™ 3,3'-Diaminobenzidine tablets	(Mat. No. D4418-5SET; D4418-50SET)
SIGMAFAST™ BCIP®/NBT, tablet	(Mat. No. B5655-5TAB; B5655-25TAB)

- Consistent, reliable quality
- Application tested for formalin-fixed and paraffin-embedded tissue sections
- Best-in-class, together with IHC-validated Prestige Antibodies®

To order, please contact your local sales office and quote Promo Code: **Y42**
Web ordering: sigma-aldrich.com/leuvoucher

Promotion is valid until June 30th 2009.

Certified Stains for Histochemistry



Hematoxylin	<i>(Mat. No. H3136)</i>
Methylene blue	<i>(Mat. No. M9140)</i>
Alizarin Red S	<i>(Mat. No. A5533)</i>
Alcian Blue 8GX	<i>(Mat. No. A3157)</i>
Sudan Black B	<i>(Mat. No. 199664)</i>
Congo Red	<i>(Mat. No. 860956)</i>
Cresyl Violet acetate	<i>(Mat. No. C5042)</i>
Eosin Y disodium salt	<i>(Mat. No. E4382)</i>
Crystal Violet	<i>(Mat. No. C0775)</i>

**33 %
Discount**

- 55 dyes with guaranteed performance
Certified by the independent Biological Stain Commission (BSC)
- Commission certification is reliability
Only those passing minimum assay limits, and who work in specified routine staining procedures, are certified
- Commission certification is cost-effective
Commission certified stains are often less expensive than non-certified

To order, please contact your local sales office and quote Promo Code: **Y42**
For a complete list, please go to sigma.com/handh

Promotion is valid until June 30th 2009.

Silver Stain (modified GMS)

Introduction

The Sigma-Aldrich Silver Stain kit is intended for use in the histologic demonstration of fungi, basement membrane and some opportunistic organisms. The Silver Stain kit is for "In Vitro Diagnostic Use". Silver methenamine-borate procedures are well documented. Generally, these require elaborate solution preparation prior to test performance. Solution stability is limited and results vary remarkably due to the capricious nature of metal impregnation and photographic development.

Sigma-Aldrich now offers a Silver Stain that incorporates a stable working silver methenamine salt along with buffer, toning reagent and developer. This provides the laboratory a unique package for visualising fungi, basement membrane and opportunistic organisms such as *Pneumocystis carinii*. Included are silver techniques for rapid staining in microwave ovens. In brief, microbial cell wall and basement membrane polysaccharides are oxidised to aldehydes by treatment with periodic acid. The aldehyde group, at alkaline pH, reduces silver ion to metallic silver. Rinsing with gold salts forms a more stable gold complex and excess silver is removed by a thiosulphate wash.

Reagents

Periodic Acid Solution Cat. no. HT 100-1, Periodic acid, 1 g/dl, in deionised water

Borax Solution Cat. no. HT 100-2, Borax, 5 g/dl, in deionised in water

Silver Methenamine Reagent Cat. no. HT 100-3, Silver methenamine, 110 mg/vial

Gold Chloride Solution Cat. no. HT 100-4, Gold chloride, 200 mg/dl, in deionised water

Sodium Thiosulphate Solution Cat. no. HT 100-5, Sodium thiosulphate, 2 g/dl, in deionised water

Reagent Preparation

Prepare working Silver Methenamine solution by combining 8 mL Borax solution, 100 mL deionised water and contents of Silver Methenamine reagent vial. Mix until dissolved. Use once, then discard.

Note:

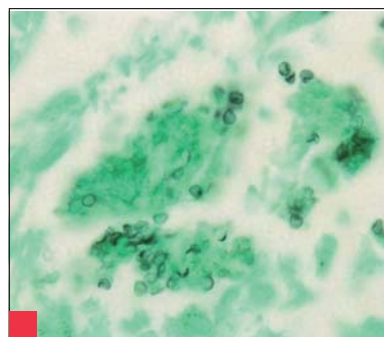
To increase tests vial to two, Silver Methenamine may be dissolved in 100 mL of deionised water and stored tightly capped. Stable refrigerated (2–8 °C) for 1 month. When ready to use, take 50 mL of Silver Methenamine solution and add 4 mL of Borax solution. Return unused portion of Silver Methenamine solution to refrigerator. Pre-warm all reagents to room temperature before use.

Silver Stain (modified GMS)

Procedure

1. Place working Silver Methenamine solution in 62 °C water bath.
2. Deparaffinise tissue sections and hydrate to deionised water.
3. Place in periodic acid solution for 5 minutes for fungi or 11 minutes for basement membrane.
4. Rinse in 6 changes of deionised water.
5. Place slides in working Silver Methenamine solution. Examine after 20 minutes for fungi and opportunistic organisms (should be dark brown) or 30 minutes for basement membrane (should be black). Return slides to working Silver Methenamine solution until desired intensity achieved.
6. Rinse in room temperature deionised water 6 times.
7. Tone sections in Gold Chloride solution for 30 seconds.
8. Rinse with room temperature deionised water 6 times.
9. Place sections in Sodium Thiosulphate solution for 2 minutes.
10. Wash well in running tap water.
11. Counterstain according to personal preference.

Results



Fungi	Purplish-brown to black
Pneumocystis	Purplish-brown to black
Basement Membrane	Black
Background	Light green or yellow (depending on counterstain)

Description

Silver Stain Kit, modified GMS

Cat. no.

HT100A



Silver Stain (modified Steiner)

Introduction

Sigma-Aldrich Silver Stain, Modified Steiner-Steiner, is intended for the demonstration of spirochetes and nonfilamentous bacteria in sections of paraffin-embedded tissue. Silver Stain reagents are for "In Vitro Diagnostic Use". Documented procedures for the demonstration of spirochetes and other opportunistic organisms include Warthin-Starry, Dieterle and Steiner-Steiner. These procedures frequently require elaborate solution preparation, and results can vary remarkably.

Sigma-Aldrich method is a modification of the Steiner-Steiner method including a microwave application to accelerate and accentuate the silver stain in tissue sections. The heat produced in the microwave oven facilitates impregnation of silver nitrate into the tissue sections, often resulting in a much cleaner background than the traditional method. Rinse in tap water and let air dry, or blot completely dry. The Sigma-Aldrich modified Steiner-Steiner procedure yields consistent, reproducible staining results.

Reagents

Uranyl Nitrate Cat. no. HT 101-1, Uranyl Nitrate, 1 % in water

Silver Nitrite Solution Cat. no. HT 101-2, Silver Nitrite, 1 % in water

Gum Mastic Solution, Alcoholic Cat. no. HT 101-3, Gum Mastic in absolute alcohol

Hydroquinone Tablets Cat. no. HT 101-4, Hydroquinone, 0.5 g, with excipients

Reagent Preparation

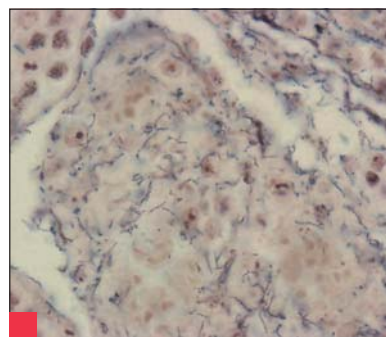
Prepare working Hydroquinone solution by dissolving one hydroquinone tablet in 25 mLs of distilled water. Mix well. Prepare Reducing solution by mixing 10 mL Gum Mastic solution, 25 mL working Hydroquinone, and 5 mL Absolute alcohol. Filter solutions. Discard Uranyl Nitrate, Silver Nitrate and Gum Mastic solutions if turbidity develops.

Silver Stain (modified Steiner)

Procedure

1. Preheat 40 mL of Uranyl Nitrate solution and 40 mL Silver Nitrate solution to 60 °C.
2. Deparaffinise tissue sections and hydrate to deionised water.
3. Place in Uranyl Nitrate solution in 60 °C water bath for 15 minutes.
4. Rinse in 3 changes of deionised water.
5. Place in Silver Nitrate solution in 60 °C water bath for 1.5 hours.
6. Rinse in 3 changes of deionised water.
7. Rinse in 2 changes each of 95 % and absolute alcohol.
8. Place in Gum Mastic solution for 5 minutes.
9. Remove slide from Gum Mastic solution and allow sections to air dry.
10. Prewarm with reducing solution to 45 °C in water bath.
11. Place in Reducing solution and incubate for approximately 25 minutes.
12. Remove coplin jar from water bath and allow slides to stand in Reducing solution at room temperature until desired stain intensity is achieved, 1-2 minutes is optional. Longer staining is optimal, if desired.
13. Dehydrate in alcohol, clear in xylene and mount.

Results



Spirochete	Brownish black
Helicobacter	Brownish black
Background	Bright yellow to light brown

Description	Cat. no.
Silver Stain Kit, modified Steiner	HT101A

SIGMA
Life Science

Stains for *In-Vitro* Diagnostic Use



Routine Stains for *In-Vitro* diagnostic use

Hematoxylin Solution, Harris Modified	(Mat. No. HHS32-1L; HHS80-2.5L)
Papanicolaou Stain, OG-6	(Mat. No. HT40132-1L; HT40180-2.5L)
Papanicolaou Stain, EA 50	(Mat. No. HT40332-1L; HT40380-2.5L)
Hematoxylin Solution, Gill No. 2	(Mat. No. GHS232-1L; GHS280-2.5L)
Hematoxylin Solution, Gill No. 3	(Mat. No. GHS332-1L; GHS380-2.5L)
Hematoxylin Solution, Mayer's	(Mat. No. MHS32-1L; MHS80-2.5L)

33 %
Discount

- More than 205 products have been CE marked according to the IVD Directive (98/79/EC)
- Save time and money with quality stains from Sigma-Aldrich
- Get consistent, reproducible results by using IVD stains from Sigma-Aldrich

For ordering please contact your local sales office and quote Promo Code: **X65**
Web ordering: sigma-aldrich.com/leuvoucher

Promotion is valid until June 30th 2009.

Increase Bioanalytical Assay Speed via Phospholipid Removal Using HybridSPE™-Precipitation Technology

Craig Aurand and An Trinh an.trinh@sial.com

Introduction

One of the primary concerns when analysing small molecules in biological samples via LC-MS/MS is ion-suppression. This is caused by one or more interfering components or species that co-elute with analyte(s) of interest during LC-MS analysis and manifests itself as loss of analyte response. These co-eluting species can affect droplet formation or ionise concurrently, resulting in an erroneous decrease (suppression) in signal response. As a result, the phenomenon often leads to poor assay reproducibility, accuracy and sensitivity. Such deleterious effects are often most notable at the lower limits of quantitation (LLOQ) [1].

Ion-suppression can be caused by a variety of agents including phthalates and plasticisers from plastic ware; common buffers and salts such as TFA; stabilisers (glycerol) and dosing agents (PEG); and plasma anti-coagulants such as lithium-heparin and sodium citrate. One of the principle causes of ion-suppression in bioanalysis is the presence of serum albumin and phospholipids during LC-MS/MS analysis in the positive ion electrospray mode (+ESI). Phospholipids are present in biological plasma at extremely high concentrations (~1 mg/mL) and can suppress analyte response if not removed during sample prep and/or resolved during chromatography [2–4].

In general, ion-suppression typically occurs during the early and late regions of analysis during reversed-phase chromatography. It is presumed that late elution ion-suppression species are predominantly caused by phospholipid contamination [4]. As researchers strive to reduce analytical run time (< 5 min.) using ballistic gradients and UPLC conditions, the risk of ion-suppression increases considerably [5]. At such ballistic gradients and fast run times, phospholipid contaminants can accumulate on the column and potentially elute uncontrollably during a given run sequence. In **Figure 1**, blank rat plasma was subjected to protein precipitation by combining rat plasma with 1 % formic acid in acetonitrile (1:3, v.v) followed by centrifugation. The resulting supernatant was analysed by LC-MS using a sub 2 µm C18 column monitoring for m/z 184 (the phosphate moiety or polar head group of phosphatidylcholine). From the chromatogram, we see that there is a steady accumulation of phospholipids on the column with each successive injection.

In Reporter 33, we introduced HybridSPE-Precipitation (HybridSPE-PPT) technology for targeted removal of phospholipids and precipitated proteins for subsequent LC-MS analysis, in which we compared its performance against traditional protein precipitation and SPE for the extraction of clenbuterol and clonidine from plasma. In this report we describe how, by removing phospholipids via HybridSPE-PPT, researchers can reduce ion-suppression, prep samples using a 2–3 step procedure, and reduce analytical run time while implementing isocratic conditions.

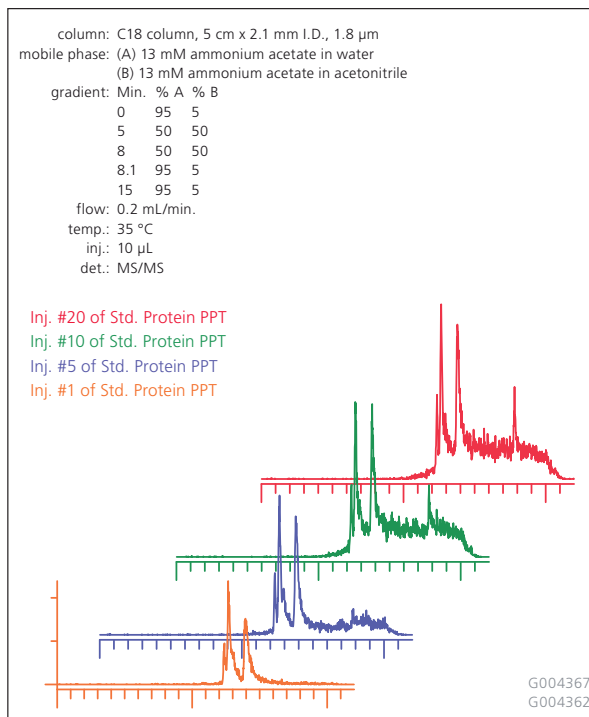


Figure 1: Accumulation of Phospholipids on LC Column after Standard Protein PPT of Plasma

How does HybridSPE-PPT work?

HybridSPE-PPT technology is a simple and generic sample prep platform designed for the gross level removal of endogenous protein and phospholipid interferences from biological plasma and serum prior to LC-MS or LC-MS/MS analysis. Biological plasma or serum is first subjected to protein precipitation via the addition and mixing of acidified (with formic acid) acetonitrile. Precipitated proteins are then removed by centrifugation and the resulting supernatant is loaded on the HybridSPE-PPT cartridge that acts as a chemical filter that specifically targets the removal of endogenous sample phospholipids.

The HybridSPE-PPT 96-well version contains a series of low porosity hydrophobic filters/frits; the packed-bed filter/frit assembly acts as a depth filter facilitating the concurrent removal of both phospholipids and precipitated proteins during the extraction process. As a result, plasma can be first added to the well plate (upper PTFE frit keeps plasma from dripping through prematurely) followed by acidified acetonitrile (precipitating agent). After a brief mixing/vortexing step, vacuum is applied to the HybridSPE-PPT plate and the resulting filtrate/eluate can be analysed directly. The phospholipid retention mechanism is based on a highly selective Lewis acid-base

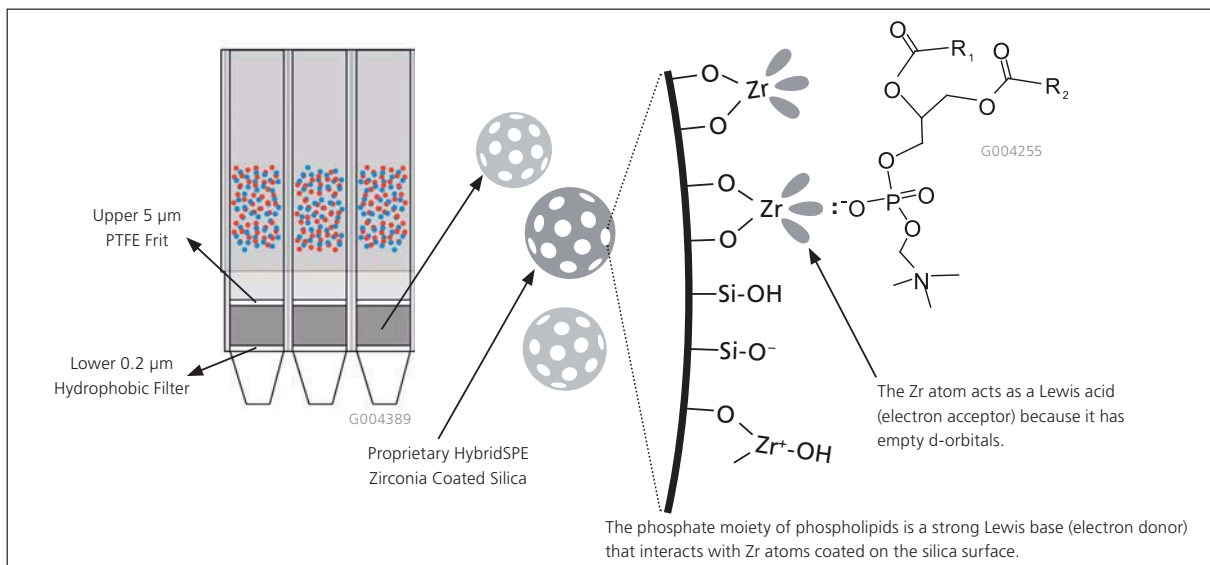


Figure 2: HybridSPE-PPT 96-well Schematic and Phospholipid Retention Mechanism

interaction between the proprietary zirconia ions functionally bonded to the HybridSPE™ stationary phase and the phosphonate moiety consistent with all phospholipids. The resulting eluent is ready for immediate LC-MS or LC-MS/MS analysis. **Figure 2** visually depicts the HybridSPE-PPT 96-well plate.

HybridSPE-PPT – 100 % Removal of Phospholipids

In this study, 100 µL of blank rat plasma was applied to a HybridSPE-PPT 96-well plate followed by 300 µL 1 % formic acid in acetonitrile. The plate was vortexed briefly and vacuum was applied. The resulting filtrate/eluate was analysed directly using the analytical conditions described in **Figure 1**. The results are depicted in **Figure 3**. By processing biological plasma samples using the HybridSPE-PPT approach, complete removal of phospholipids was achieved. Unlike standard protein precipitation (**Figure 1**), no accumulation of phospholipids was observed using the HybridSPE-PPT

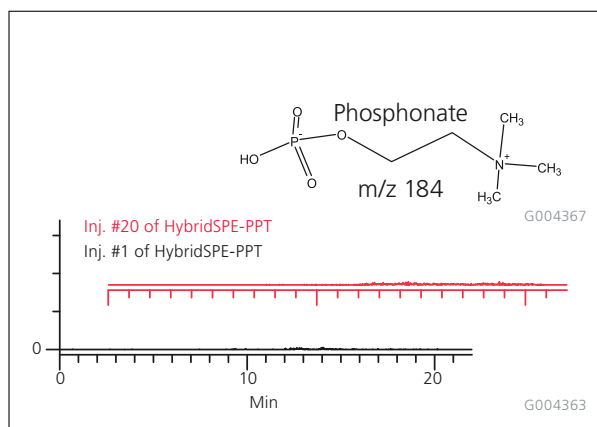


Figure 3: No Accumulation of Phospholipids Using HybridSPE-PPT

approach. In addition, because the HybridSPE-PPT 96-well acts as both a depth filter and a chemical filter to remove particulate matter and phospholipids concurrently, backpressure was significantly reduced (**Figure 4**).

Reduce LC-MS Run Time using HybridSPE-PPT

In this study, plasma samples spiked with verapamil, normethyl verapamil, and methoxy verapamil at the level of 10 ng/mL were subjected to protein precipitation by diluting the samples with 1 % formic acid in acetonitrile (1:3, v/v) and analysed by LC-TOF/MS (**Figure 5**). From these results, we see that although verapamil and its metabolites are eluted early in the run (~ 90 sec.), contaminating phospholipids are strongly retained and do not elute off from the column until after 10 min. Therefore, shorter run times (e.g. < 5 min.) pose a great risk to on-column phospholipid accumulation/contamination.

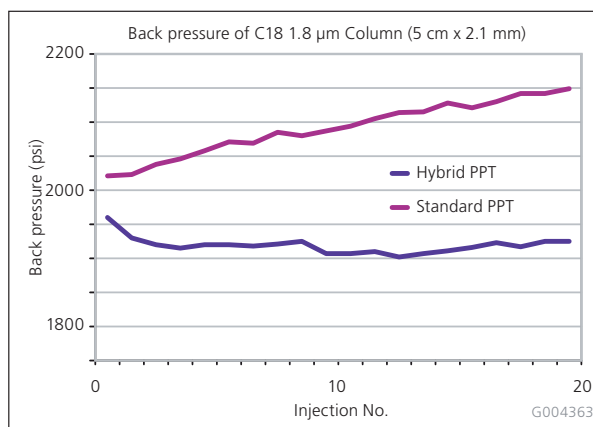


Figure 4: Stabilisation of Backpressure Using HybridSPE-PPT

Because HybridSPE™-PPT depletes phospholipids from the plasma sample (**Figure 3**), it is not necessary to run long gradient conditions to “wash off” contaminating phospholipids evident when using alternative sample prep techniques. Rat plasma spiked with verapamil and metabolites (10 ng/mL) were extracted using the HybridSPE-PPT method described for **Figure 3** and analysed by LC-TOF/MS using Ascentis Express C18. In **Figure 6**, we demonstrate the utility of combining HybridSPE-PPT and Ascentis Express column technology. Less than 90-second run-time was achieved under isocratic conditions; and because HybridSPE-PPT was employed during sample prep, risk of phospholipid ion-suppression and column accumulation was eliminated.

Conclusion

In this report, we discussed the ion-suppression impact of phospholipids and how traditional sample prep techniques such as protein precipitation do not remove this common matrix interference. As researchers strive for shorter run-times using ballistic gradients, the risk of column phospholipid accumulation grows considerably. By using HybridSPE-PPT, the risk of phospholipid contamination is eliminated. As a result, shorter run times are possible. As a demonstration, HybridSPE-PPT was combined with Ascentis Express C18 technology for the extraction and analysis of verapamil (and metabolites) from plasma. By combining the two techniques, less than 90-second run-time was achieved under isocratic conditions.

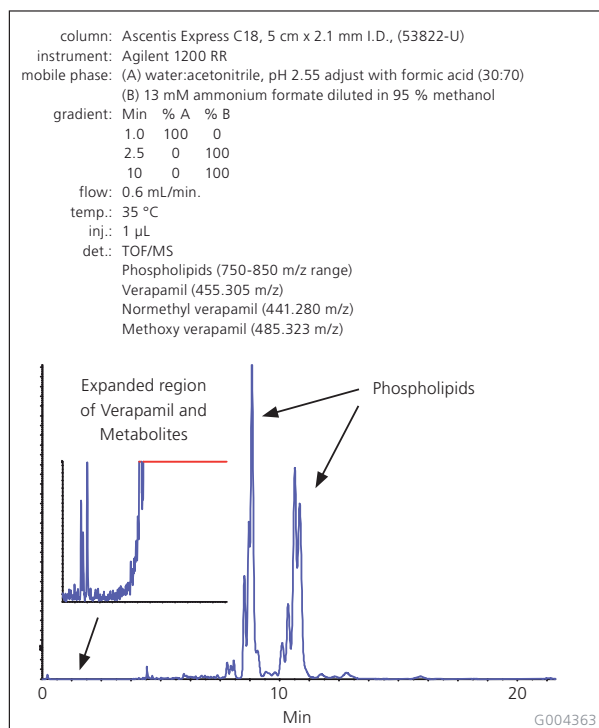


Figure 5: Phospholipid Contamination from Standard Protein PPT Requires Increased Run Time (> 10 min.)

Conditions identical to Figure 5, except for mobile phase
 Mobile phase: water:acetonitrile, pH 2.55 adjust with formic acid (30:70) (isocratic)

1. Normethyl verapamil
2. Verapamil
3. Methoxyverapamil

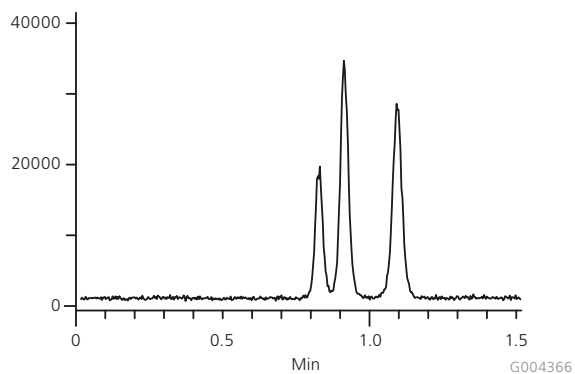


Figure 6: Less than 90-Sec. Run-Time Achieved Using Ascentis Express C18 and HybridSPE-PPT for Verapamil and Metabolites in Rat Plasma

References

- 1] King et al., J Am Soc Mass Spectrom 11 (2000), 942–50.
- 2] Ahnoff et al., Proceedings of the 51st ASMS Conference on Mass Spectrometry and Allied Topics Montreal, Canada (2003).
- 3] Little et al., Journal of Chrom B, 833 (2006), 219–230.
- 4] Shen et al., Journal of Pharmaceutical and Biomedical Analysis 37 (2005) 359–367.
- 5] Xu et al., Journal of Pharmaceutical and Biomedical Analysis 44 (2007) 342–355.

Featured Products

Description	Cat. no.
HybridSPE-Precipitation	
96-well Plate, 50 mg/well, pk. 1	575656-U
Cartridge, 30 mg/1 mL, pk. 100	55261-U
Ascentis Express C18	
5 cm x 2.1 mm I.D., 2.7 µm	53822-U

Related Information

For more information on HybridSPE-Precipitation and Ascentis Express Column Technology, please visit sigma-aldrich.com/hybrid-spe-ppt and sigma-aldrich.com/express, respectively. For test samples of the HybridSPE please contact our technical service.

NEW! HybridSPE™ – Precipitation Technology

SUPELCO
Analytical



Removal of Proteins and Phospholipids for LC-MS

Combines the simplicity of protein precipitation and the selectivity of SPE for the targeted removal of phospholipids and proteins from biological samples like plasma and serum

Facts, Features & Benefits:

Merges Protein Precipitation & SPE

- 100 % Removal of Proteins & Phospholipids in one simple 2-3 Step Procedure (96-well format)
- Minimal to no Method Development

No interference from phospholipids in LC-MS leads to

- Cleaner Samples
- Less Ion Suppression
- More reproducible Results & Sensitivity
- Longer Column Life

Patent pending and Award winning* technology based on zirconia-coated silica available in 96-well plates and 1 mL SPE tubes.

For further information on the HybridSPE-ppt please visit us at sigma-aldrich.com/hybridspe-ppt. If you would like to try the HybridSPE-ppt, request your sample of the 1 mL HybridSPE cartridges from our technical service.

***Best New Separations Product
in 2008**



Scientist Choice Awards 2009 of the selectscience.net community

Our Innovation, Your Research – Shaping the Future of Life Science

SIGMA
Life Science

Bacillus: An Ubiquitous Bacteria Genus

Some *Bacillus* species play a critical role in helping us understand more about bacteria, while others are dangerous spoiling organisms or even pathogens. All, however, are important in biotechnological research.

Jvo Siegrist, Product Manager Microbiology ivo.siegrist@sial.com

Bacillus species are Gram-positive, rod-shaped bacteria; they can be either obligate or facultative aerobes and show positive reaction in the catalase test. Members of the genus *Bacillus* are known to form spores under stressful conditions. These endospores are highly resistant to heat and radiation and are viable for extremely long periods.

Kingdom: Bacteria
Division: Firmicutes
Class: Bacilli
Order: Bacillales
Family: Bacillaceae
Genus: *Bacillus*

B. subtilis is one of the best-understood bacteria, thus it is often used in molecular biology and as a general model organism. Its harmless nature, brilliant genetic amenability and relatively large gene size make the organism a highly valuable tool for science and demonstration purposes. *Bacillus subtilis* has been used to help demonstrate biochemical differentiation, gene/protein regulation and cell cycle events in bacteria.

B. thuringiensis is an important insect pathogen. Its Bt toxin is specifically active against several undesirable species of insects. Therefore, the Cry and Cyt genes are used for the production of biological-based insecticides; in agriculture, the toxin genes are used to modify crops to make them insect-resistant. When insects take up the substance, it is cleaved under the alkaline conditions in their digestive tract, and the toxin becomes active. The protein then inserts itself into the insect's gut cell membranes, forming a pore and resulting in swelling, cell lysis and possible death for the insect.

B. anthracis causes anthrax. The name *anthracis* comes from the Greek *anthrakis*, meaning "coal", because in the most common form of the disease, cutaneous anthrax, the sufferer develops large black skin lesions. It is a relatively big (1–6 µm) facultative aerobe, non-motile organism that can form centrally located ellipsoid spores even under stressful conditions. The cells are usually built in chains. In vivo, the bacterium forms a capsule from polyglutamate, which protects it from phagocytosis. On blood agar cultures, however, the capsule is usually not present. Genotypically and phenotypically, it is very similar to *Bacillus cereus* and to *Bacillus thuringiensis*, but there are some differentiating characteristics. (Table 1)

Did you know ...

that *B. subtilis* was involved in the testing of the New York subway system's vulnerability to a biological attack?

The organism was released to find out how many people would be killed in the event of a bio-warfare attack. The result? The entire system could be contaminated by the release of bacteria in just one train.

Characteristic	<i>B. anthracis</i>	<i>B. cereus</i> and <i>B. thuringiensis</i>
Growth requirement for thiamin	+	-
Hemolysis on sheep blood agar	-	+
Glutamyl-polypeptide capsule	+	-
Lysis by gamma phage	+	-
Motility	-	+
Growth on chloral hydrate agar	-	+
String-of-pearls test	+	-

Table 1: Differentiating characteristics of *B. anthracis*, *B. cereus* and *B. thuringiensis*

Brand	Cat. no.	Name	Description
Fluka	55678	PLET Agar Anthraxis- Selective- Supplement (Fluka 72659)	The best selective medium for isolation and cultivation of <i>Bacillus anthracis</i> from environmental specimens, animal products or clinical specimens, inhibiting <i>Bacillus cereus</i> .
Fluka	70133	Blood Agar (Base)	A non-selective medium for the isolation and cultivation of many pathogenic and non-pathogenic microorganisms.

Table 2: Media for detection and differentiation of *B. anthracis*

B. cereus causes a foodborne illness similar to those caused by *Clostridium perfringens* or *Staphylococcus aureus*. *B. cereus* is a facultative aerobe, beta hemolytic soil bacteria that produces exotoxins. There are two types of illness caused by *B. cereus*, depending on the contaminated substance ingested: a diarrhoea type (similar to that caused by *C. perfringens*) and a vomiting type (similar to that caused by *Staphylococcus aureus*). The minimum

infectious dose is about 10^6 germs/g. Infection sources for the diarrhoea type of *B. cereus* infection are sweets (pudding, vanilla sauce), meats (roast, goulash, sausages), vegetables, salads, soups and UHT milk products. The vomiting type of *B. cereus* illness is caused by contaminated cooked rice that has been reheated. The risk for strong propagation and the resulting illness can be minimised by storing foods at <5 °C or >65 °C and by rapidly cooling down foods, thus lowering the pH value to <4.5 with a respective aw-value of <0.95 . Note: *B. cereus* spores are in most cases not eliminated by heating; in fact, heating activates spore germination. At the same time, however, the spoilage flora is eliminated.

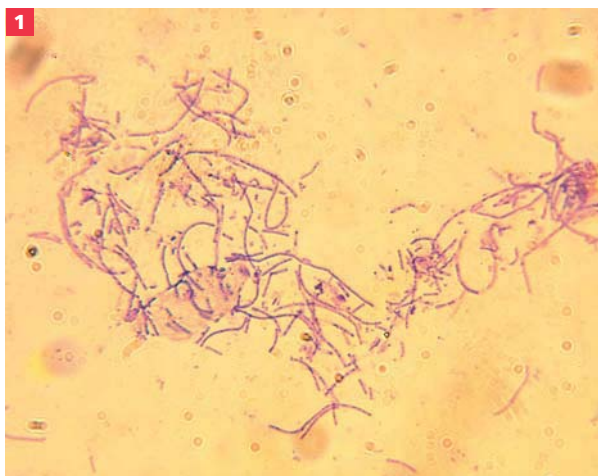


Figure 1: Microscopic picture from *Bacillus anthracis*

Non-selective media for *Bacillus* species

Brand	Cat. no.	Description
Fluka	70181	Antibiotic Agar No. 1
Fluka	70182	Antibiotic Agar No. 2
Fluka	22089	Casein peptone Lecithin Polysorbate Broth
Fluka	22095	CASO Agar
Fluka	22098	CASO Broth
Fluka	70185	Czapek Dox Agar
Sigma	D3435	Dey-Engley Neutralising Broth
Fluka	70147	Milk Agar
Fluka	72548	Nitrate Broth
Fluka	70148	Nutrient Agar
Fluka	17179	Nutrient Agar pH 6.0 with 0.8 % NaCl
Fluka	44776	Nutrient Agar Plates (Diameter 55 mm)
Fluka	03856	Nutrient Broth No. 4
Fluka	70179	Peptone Water
Fluka	70152	Plate Count Agar
Fluka	88588	Plate Count Agar according to Buchbinder et al.
Fluka	68414	Plate Count Agar according to Buchbinder et al.
Fluka	00464	Plate Count Agar Plates (diameter 55 mm)

Table 3: Medium for *Bacillus* species

Non-selective media for *Bacillus* species (continuation)

Brand	Cat. no.	Description
Fluka	19718	Plate Count Agar, Vegetone
Fluka	80957	Plate Count Skim Milk Agar
Fluka	17175	Skim Milk Agar, modified
Fluka	17274	Thermoacidurans Agar
Fluka	70157	Thioglycollate Broth (USP Alternative)
Fluka	90404	Thioglycollate Broth with Resazurine
Fluka	28976	Thioglycollate Medium with K Agar
Fluka	79872	Tryptic Soy Agar
Fluka	22091	Tryptic Soy Agar
Fluka	57994	Tryptic Soy Agar Plates (Diameter 55 mm)
Fluka	14432	Tryptic Soy Agar, Vegetone
Fluka	22092	Tryptic Soy Broth
Fluka	43592	Tryptic Soy Broth
Fluka	51228	Tryptic Soy Broth No. 2
Fluka	41298	Tryptic Soy Broth, Vegetone
Fluka	51414	Tryptic Soya Agar with Polysorbate 80 and Lecithin
Fluka	70159	Tryptone Glucose Extract Agar
Sigma	T2188	Tryptone Glucose Yeast Extract Agar

Non-selective differential media for *Bacillus* species

Brand	Cat. no.	Description
Fluka	70133	Blood Agar (Base)
Fluka	39212	Blood Agar SLMB
Fluka	21065	Calcium caseinate Agar
Fluka	70136	Deoxyribonuclease Test Agar
Fluka	30787	Deoxyribonuclease Test Agar
Fluka	31415	Dextrose Caseinpeptone Agar
Sigma	G0289	Gelatin Iron Agar
Fluka	16447	Glucose Bromcresol Purple Agar
Sigma	M1928	MYP Agar Base
Fluka	70151	Nutrient Gelatin
Fluka	91015	Tributyryn Agar

Selective differential media for *Bacillus* species

Brand	Cat. no.	Description
Fluka	22310	Cereus Selective Agar
Fluka	92325	HiCrome™ <i>Bacillus</i> Agar

Detection of trace amounts of penicillin in milk using *Bacillus stearothermophilus*

Brand	Cat. no.	Description
Fluka	17186	PM Indicator Agar

Products for the identification and differentiation of *Bacillus* species

Brand	Cat. no.	Description
Fluka	88597	Catalase Test
Fluka	77730	Gram Staining Kit
Fluka	04551	Schaeffer and Fulton Spore Stain Kit
Fluka	44378	M'Fadyean Stain Solution

Table 4: Products for the identification and differentiation of *Bacillus* species

TRADEMARKS: HybridSPE, SIGMAFAST, HiCrome

registered TRADEMARKS: Prestige Antibodies, Histopaque, Percoll, Ficoll, ExtrAvidin, HiCrome

Argentina

SIGMA-ALDRICH DE ARGENTINA S.A.
 Free Tel: 0810 888 7446
 Tel: (+54) 11 4556 1472
 Fax: (+54) 11 4552 1698

Australia

SIGMA-ALDRICH PTY LTD.
 Free Tel: 1800 800 097
 Free Fax: 1800 800 096
 Tel: (+61) 2 9841 0555
 Fax: (+61) 2 9841 0500

Austria

SIGMA-ALDRICH HANDELS GmbH
 Tel: (+43) 1 605 81 10
 Fax: (+43) 1 605 81 20

Belgium

SIGMA-ALDRICH NV/SA.
 Free Tel: 0800 14747
 Free Fax: 0800 14745
 Tel: (+32) 3 899 13 01
 Fax: (+32) 3 899 13 11

Brazil

SIGMA-ALDRICH BRASIL LTDA.
 Free Tel: 0800 701 7425
 Tel: (+55) 11 3732 3100
 Fax: (+55) 11 5522 9895

Canada

SIGMA-ALDRICH CANADA LTD.
 Free Tel: 1800 565 1400
 Free Fax: 1800 265 3858
 Tel: (+1) 905 829 9500
 Fax: (+1) 905 829 9292

China

SIGMA-ALDRICH (SHANGHAI)
 TRADING CO. LTD.
 Free Tel: 800 819 3336
 Tel: (+86) 21 6141 5566
 Fax: (+86) 21 6141 5567

Czech Republic

SIGMA-ALDRICH spol. s r. o.
 Tel: (+420) 246 003 200
 Fax: (+420) 246 003 291

Denmark

SIGMA-ALDRICH DENMARK A/S
 Tel: (+45) 43 56 59 10
 Fax: (+45) 43 56 59 05

Finland

SIGMA-ALDRICH FINLAND OY
 Tel: (+358) 9 350 9250
 Fax: (+358) 9 350 92555

France

SIGMA-ALDRICH CHIMIE S.à.r.l.
 Free Tel: 0800 211 408
 Free Fax: 0800 031 052
 Tel: (+33) 474 82 28 00
 Fax: (+33) 474 95 68 08

Germany

SIGMA-ALDRICH CHEMIE GmbH
 Free Tel: 0800 51 55 000
 Free Fax: 0800 64 90 000
 Tel: (+49) 89 6513 0
 Fax: (+49) 89 6513 1160

Greece

SIGMA-ALDRICH (O.M.) LTD.
 Tel: (+30) 210 994 8010
 Fax: (+30) 210 994 3831

Hungary

SIGMA-ALDRICH Kft
 Ingyenes zöld telefon: 06 80 355 355
 Ingyenes zöld fax: 06 80 344 344
 Tel: (+36) 1 235 9055
 Fax: (+36) 1 235 9050

India

SIGMA-ALDRICH CHEMICALS
 PRIVATE LIMITED
 Telephone
 Bangalore: (+91) 80 6621 9600
 New Delhi: (+91) 11 4165 4255
 Mumbai: (+91) 22 2570 2364
 Hyderabad: (+91) 40 4015 5488
 Fax
 Bangalore: (+91) 80 6621 9650
 New Delhi: (+91) 11 4165 4266
 Mumbai: (+91) 22 2579 7589
 Hyderabad: (+91) 40 4015 5466

Ireland

SIGMA-ALDRICH IRELAND LTD.
 Free Tel: 1800 200 888
 Free Fax: 1800 600 222
 Tel: (+353) 1 404 1900
 Fax: (+353) 1 404 1910

Israel

SIGMA-ALDRICH ISRAEL LTD.
 Free Tel: 1 800 70 2222
 Tel: (+972) 8 948 4100
 Fax: (+972) 8 948 4200

Italy

SIGMA-ALDRICH S.r.l.
 Numero Verde: 800 827018
 Tel: (+39) 02 3341 7310
 Fax: (+39) 02 3801 0737

Japan

SIGMA-ALDRICH JAPAN K.K.
 Tel: (+81) 3 5796 7300
 Fax: (+81) 3 5796 7315

Korea

SIGMA-ALDRICH KOREA
 Free Tel: (+82) 80 023 7111
 Free Fax: (+82) 80 023 8111
 Tel: (+82) 31 329 9000
 Fax: (+82) 31 329 9090

Malaysia

SIGMA-ALDRICH (M) SDN. BHD
 Tel: (+60) 3 5635 3321
 Fax: (+60) 3 5635 4116

Mexico

SIGMA-ALDRICH QUÍMICA, S.A. de C.V.
 Free Tel: 01 800 007 5300
 Free Fax: 01 800 712 9920
 Tel: (52) 722 276 1600
 Fax: (52) 722 276 1601

The Netherlands

SIGMA-ALDRICH CHEMIE BV
 Free Tel: 0800 022 9088
 Free Fax: 0800 022 9089
 Tel: (+31) 78 620 5411
 Fax: (+31) 78 620 5421

New Zealand

SIGMA-ALDRICH NEW ZEALAND LTD.
 Free Tel: 0800 936 666
 Free Fax: 0800 937 777
 Tel: (+61) 2 9841 0555
 Fax: (+61) 2 9841 0500

Norway

SIGMA-ALDRICH NORWAY AS
 Tel: (+47) 23 17 60 60
 Fax: (+47) 23 17 60 50

Poland

SIGMA-ALDRICH Sp. z o.o.
 Tel: (+48) 61 829 01 00
 Fax: (+48) 61 829 01 20

Portugal

SIGMA-ALDRICH QUÍMICA, S.A.
 Free Tel: 800 202 180
 Free Fax: 800 202 178
 Tel: (+351) 21 924 2555
 Fax: (+351) 21 924 2610

Russia

SIGMA-ALDRICH RUS, LLC
 Tel: (+7) 495 621 6037
 (+7) 495 621 5828
 Fax: (+7) 495 621 5923

Singapore

SIGMA-ALDRICH PTE. LTD.
 Tel: (+65) 6779 1200
 Fax: (+65) 6779 1822

Slovakia

SIGMA-ALDRICH spol. s r.o.
 Tel: (+421) 255 571 562
 Fax: (+421) 255 571 564

South Africa

SIGMA-ALDRICH
 SOUTH AFRICA (PTY) LTD.
 Free Tel: 0800 1100 75
 Free Fax: 0800 1100 79
 Tel: (+27) 11 979 1188
 Fax: (+27) 11 979 1119

Spain

SIGMA-ALDRICH QUÍMICA, S.A.
 Free Tel: 900 101 376
 Free Fax: 900 102 028
 Tel: (+34) 91 661 99 77
 Fax: (+34) 91 661 96 42

Sweden

SIGMA-ALDRICH SWEDEN AB
 Tel: (+46) 8 742 4200
 Fax: (+46) 8 742 4243

Switzerland

SIGMA-ALDRICH CHEMIE GmbH
 Free Tel: 0800 80 00 80
 Free Fax: 0800 80 00 81
 Tel: (+41) 81 755 2828
 Fax: (+41) 81 755 2815

United Kingdom

SIGMA-ALDRICH COMPANY LTD.
 Free Tel: 0800 717 181
 Free Fax: 0800 378 785
 Tel: (+44) 1747 833 000
 Fax: (+44) 1747 833 313
 SAFC (UK) Free Tel: 01202 712305

United States

SIGMA-ALDRICH
 P.O. Box 14508
 St. Louis, Missouri 63178
 Toll-Free: 800 325 3010
 Toll-Free Fax: 800 325 5052
 Call Collect: (+1) 314 771 5750
 Tel: (+1) 314 771 5765
 Fax: (+1) 314 771 5757

Vietnam

SIGMA-ALDRICH Pte Ltd. VN R.O.
 133/27N Ngo Duc Ke Street
 Ward 12 Binh Thanh District
 Ho Chi Minh City
 Tel: (84) 8 3516 2810
 Fax: (84) 8 6258 4238

Internet

sigma-aldrich.com





World Headquarters

3050 Spruce St., St. Louis, MO 63103
 (314) 771-5765
sigma-aldrich.com

Technical Service EUR: EurTechServ@sial.com

Technical Service US: techservice@sial.com

Development/Bulk Manufacturing Inquiries SAFC® (800) 244-1173

© 2009 Sigma-Aldrich Co. All rights reserved. SIGMA, , SAFC, SAFC®, SIGMA-ALDRICH, ALDRICH, , FLUKA, , and SUPELCO,  are trademarks belonging to Sigma-Aldrich Co. and its affiliate Sigma-Aldrich Biotechnology, L.P. Sigma brand products are sold through Sigma-Aldrich, Inc. Sigma-Aldrich, Inc. warrants that its products conform to the information contained in this and other Sigma-Aldrich publications. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see reverse side of the invoice or packing slip.

Date: 02/2009;
 SAMS Code: LEU



*Accelerating Customers'
 Success through Innovation
 and Leadership in Life
 Science, High Technology
 and Service*

SIGMA-ALDRICH®