

Advertisement feature

Fantastic Abs

The latest developments in antibody production, purification and analysis

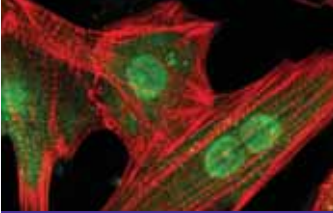


Image supplied by Rockland Immunochemicals

Research on antibodies began in 1890 and the word antibody is first recorded in Paul Ehrlich's celebrated 1891 essay 'Experimental Studies on Immunity'. Since then, extensive research has revealed the functions and the molecular structures of antibodies. Today, the ability of antibodies to recognise specific proteins means that they are used in a wide range of research applications from cell identification to immunohistochemistry.

Antibodies

There is continuous demand for antibodies that are able to recognise the three-dimensional structure of proteins. Conventional peptide derived antibodies typically only recognise sequential epitopes in the linear amino acid sequence (primary structure) of a protein. In many cases these antibodies are not able to bind to three-dimensional proteins and so the native protein structures cannot be identified using immunoprecipitation or immunohistochemistry. **Strategic Diagnostics** has developed its **Genomic Antibody Technology™ (GAT)** to tackle this issue, and has made more than 800 oncology-focused antibodies available, each recognising non-overlapping conformational (discontinuous) specific epitopes present in the three-dimensional structure of a protein.

Invitrogen, a division of **Life Technologies**, has launched the unconjugated monoclonal antibody specific to human FAK [pY397] that is validated for use in Western Blot, immunocytochemistry and immunofluorescence experiments. **Anti-FAK [pY397]** recognises the expressed product of the PTK2 gene. Another new antibody from Invitrogen is the **monoclonal anti-Abeta 42 Clone: 20-1** that is validated for immunohistochemistry and ELISA, and reacts with amyloid beta-protein (Abeta) 42 associated with neuronal injury and death in Alzheimer's disease.

Antibodies remain the most specific, readily producible tool at our disposal for research on cellular control mechanisms. This research is vital as it is often the breakdown or alteration of such control mechanisms that result in disease states. **Rockland Immunochemicals** has focused its efforts on producing monoclonal antibodies to the PI 3-kinase pathway,

which are validated by immunofluorescence microscopy, immunohistochemistry, western blotting and various high content screening assays.

Funakoshi offers a series of modified **p53 specific antibodies**. These purified monoclonal p53 antibodies are highly specific and, amongst other applications, the glycosylation-specific p53 antibodies have been used to detect DNA damage in apoptotic cells.

Recombinant antibody technology is a powerful tool, allowing rapid, large-scale antibody expression, and improvement of antibody performance, through gene modification. **RayBiotech** offers more than 100 recombinant antibodies, as well as custom antibody development services. Using the **RayBio®** custom recombinant antibody program, it is possible to isolate monoclonal antibody genes from an existing hybridoma, or even select a highly specific clone from an immunised animal (mouse, rabbit or chicken). By converting the gene to a bacterial expression system, valuable antibodies can be recovered from an unstable hybridoma cell line or host animal.

Chicken antibodies display less cross-reactivity with mammalian proteins, producing fewer false positive reactions in certain immunochemical assays. Chicken IgY is also typically more stable than IgG derived from mammalian species. **Sigma-Aldrich** has launched its first range of **IgY antibodies** with a library of 700 polyclonal IgY antibodies. The new antibodies are derived from chicken embryos and are prepared using recombinant antigens.

Endotoxins are major contaminants found in commercially available proteins, antibodies or biologically active substances, and can often complicate studies. **Leinco** has developed a technology for monoclonal antibody manufacture that results in

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The BioFlo/CelliGen[®] 115, a versatile, entry-level fermentor and bioreactor system from New Brunswick Scientific



The MabDirect MM Fixed-D disposable chromatographic column from Upfront Chromatography

functional formulations with low endotoxin levels. The new line of formulations from Leinco is therefore ideal for both *in vivo* and *in vitro* experiments.

Origene provides a range of antibodies for western blot. Each antibody is validated with an over-expression cell lysate (HEK293 cells transfected with specific tagged genes) using western blot assay. All antibodies supplied by Origene have passed this Western QC and in addition, a vial of the matching cell lysate will be included with each antibody shipment, for use in a control lane in the researchers' own western blot analyses.

Production and purification products

Unlike the common bacterial coat protein matrices (Protein A, G and L), BAC's CaptureSelect[®] Antibody Toolbox matrices are specifically designed to purify any antibody format with high affinity and specificity, using gentle elution conditions. The latest addition to the product range is the CaptureSelect Human IgG4 affinity matrix, which enables the purification of human IgG4s without cross reactivity with human IgG subclasses 1, 2, and 3. BAC's range of research products makes it easy for researchers to select the most appropriate purification media for their applications, and standardised purification protocols minimise testing and optimisation stages. "There is a real need for robust and simple affinity products that have specificity for individual antibody isotypes and subclasses," commented Laurens Sierkstra, CEO of BAC.

Upfront Chromatography's disposable expanded bed adsorption platform Rhobust[™] uses pre-packed, and pre-sanitised antibody purification columns that eliminate the need for pre-filtration and enable crude feedstock to be directly loaded into the adsorption units. The MabDirect Mixed Mode adsorbent used in the columns enables the efficient purification of monoclonal antibodies. "The flexibility offered by new disposable columns will be a major benefit for multi-product facilities such as CMOs and pharma pilot plants," said Allan Lihme, Technical Director, Upfront Chromatography. The combined

advantages of disposability and crude feedstock processing have delivered a single high-yielding system, which eliminates the requirement for both cleaning validation and further clarification steps such as filtration and centrifugation.

Custom-designed BioProcess affinity chromatography media, for the industrial scale purification of antibodies and antibody fragments, are available from GE Healthcare. The media enable the rapid one-step purification of biopharmaceuticals with both high purity and high yield. The custom media include: KappaSelect for antibody fragments with kappa light chain; IgSelect for human IgG; AVB Sepharose High Performance for AAV; and VIIISelect for Factor VIII production. The media have all been designed with the robustness necessary for use in bioprocessing, including high chemical stability when using typical CIP (Clean-In-Place) solutions, and a rigid agarose base matrix enabling both a high flow rate and the processing of large sample volumes, resulting in increased throughput.

A versatile, entry-level fermentor and bioreactor system, ideal for producing antibodies, is now available from New Brunswick Scientific. Offered in 1.3 to 14 litre capacities, the BioFlo/CelliGen[®] 115 is easy to use and takes the complexity out of equipment selection, set-up and operation.

Providing sophisticated antibody development, BioGenes has successfully developed antibody-producing cell hybrids against haptens, Botulinum toxins, cell toxins (Amanitin), mouse cytokines, unconjugated small molecules, and full size or fragmented antibodies. BioGenes has also successfully created mAbs differentiating between the mono-phosphate and the bi-phosphate form of small organic molecules, and mAbs against bi-modified peptides that are highly specific without cross-reacting with either the non-modified peptide or with peptides bearing only one of the modifications.

Analysis

The Tailing and Tagging labelling system from Dojindo makes it possible to prepare

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Laurens Sierkstra, CEO, BAC.

various conjugates using only a couple µg of antibody. The "Tailed" antibody can be "Tagged" by virtually any kind of component such as enzymes, fluorophores, chemicals, proteins and peptides. The Tailing reagent is a biotin-head molecule with a long hydrophilic spacer and an NH₂-reactive site at the end, which does not block the antibody's recognition site. The Tagging reagent is a modified streptavidin and binds irreversibly with the biotin moiety of the Tailing reagent, resulting in conjugates that are stable for several months.

Companies mentioned in this Product Focus:

BAC – www.captureselect.com
 BioGenes – www.biogenes.de
 Dojindo – www.dojindo.com
 Funakoshi – www.funakoshi.co.jp/expo
 GE Healthcare – www.gelifesciences.com
 Invitrogen – www.invitrogen.com
 Leinco – www.leinco.com
 Life Technologies – www.lifetechnologies.com
 New Brunswick Scientific – www.nbsc.com
 Origene – www.origene.com/antibody
 Rockland Immunochemicals – www.rockland-inc.com
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