Multiclonals™

TechNote

Abcalis

Anti-human IgG secondary antibodies

An animal free recombinant antibody mix for unlimited reproducibilty

The immunization of animals and the extraction of animal sera is a practice that was initially conceived by Emil von Behring in the year 1890 and has been around ever since. Today, *in vitro* technologies for antibody discovery and production allow to by-pass immunization. At Abcalis, antibody phage display is used to discover high affinity antibodies from naive antibody libraries generated from a multitude of human B cells donors (Fig. 1). All our binders are always defined by their sequence.

This further allows to convert them into various IgG formats adapted to the needs of various assays. For example, the same antibody can be provided as an IgG from mouse, goat, human, or others. All our Multiclonals™ are produced in serum-free conditions.

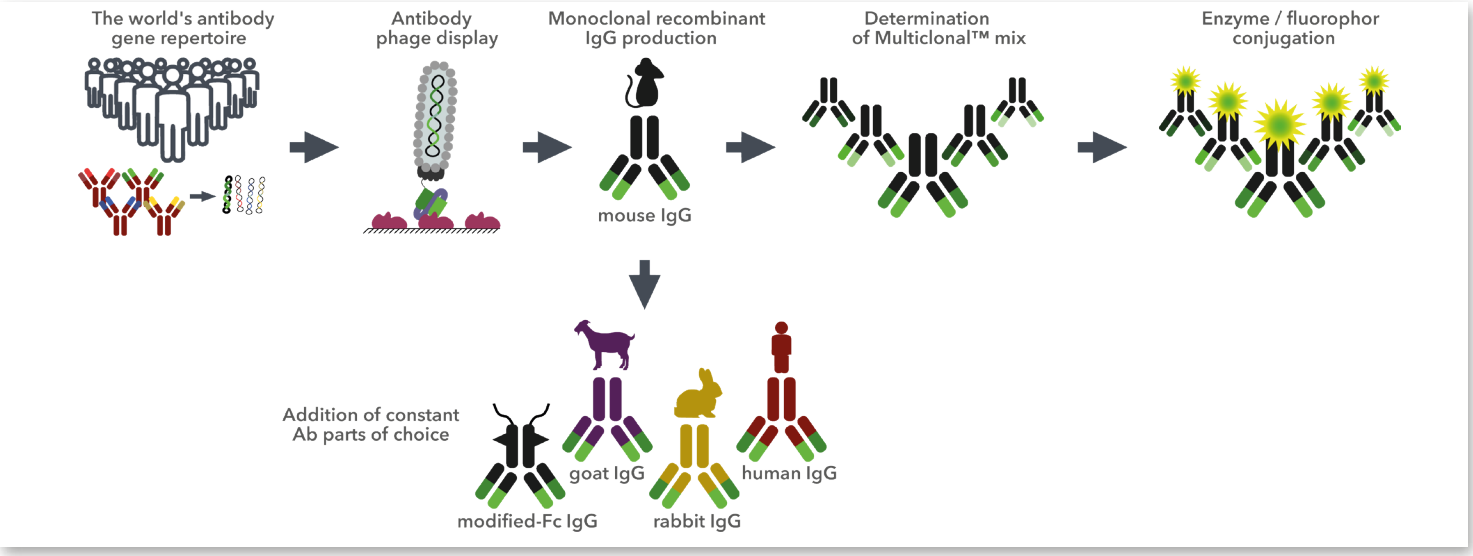

Fig. 1: How Multiclonals™ are generated by phage display.

MULTICLONALS

Advantages of Multiclonals™ for your work

First, Abcalis® Multiclonals™ (Fig. 2) contain only target specific antibodies, not a mixture of Immunoglobulins with unknown specificity, unlike all polyclonal antibodies, the major type used in research today and even unlike a significant fraction of monoclonal Hybridoma-derived „monoclonal" antibodies[1].

This leads to superior specificity and offers product customization.

Second, the antibody can never be lost, since the sequence of every Abcalis® antibody is always known right from the start. This provides scientific and regulatory reproducibility forever, which is also a critical aspect in respect of high QC standards and IVD regulations.

Third, since Abcalis® antibodies are generated via phage-display and produced entirely *in vitro* by recombinant methods, they are completely animal free. They come as highly purified Protein A protein.

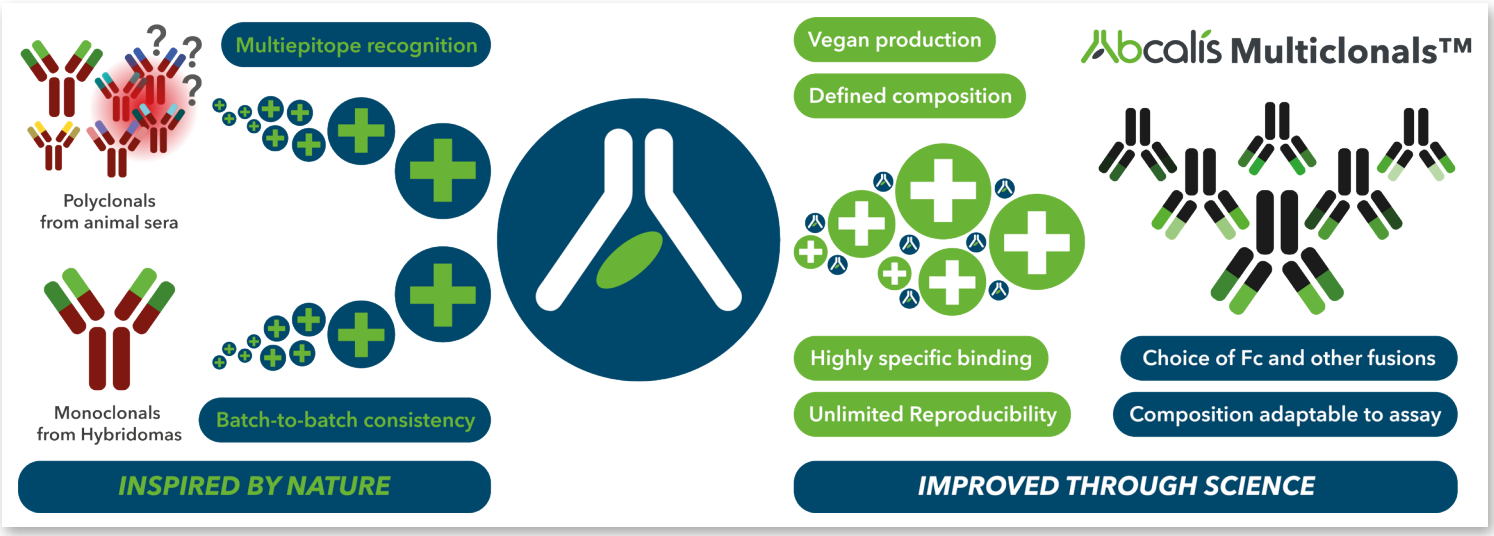

Fig. 2: Abcalis® Multiclonals™ combine the best of both worlds and share a unique set of features.

As a result, our antibodies are also free of any animal derived components like IgG or serum contaminations typically found in conventional antisera, as their production is done in defined media free of such components.

Multiclonal™ recombinant antibodies combine the best features of polyclonal antisera and monoclonal antibodies while eliminating their disadvantages. By detecting more than one epitope of the antigen, Multiclonals™ provide the sensitivity and reliability of polyclonals, but unlike polyclonals, they do not contain any additional unknown IgG. Accurately selected antibodies are combined in the mix, which are always defined by their sequence. As a result, Multiclonals™ provide unlimited reproducibility and minimized unwanted side reactivities, establishing a new quality standard for secondary antibodies. Lastly, their generation and production is entirely animal free.

[1] Bradbury et al (2018): When monocolal antibodies are monospecific: Hybridomas frequently express additional functional variable regions. MAbs 10(4):539-546

Multiclonals™ capture human IgG from serum samples

Monoclonal, polyclonal and Multiclonal™ antibodies were immobilized to capture hIgG from human serum (Fig. 3).

The comparison of anti-human Abcalis® Multiclonal™, monoclonal, and goat polyclonal serum product unveiled the limitations of the monoclonal due to single epitope detection in terms of sensitivity. This is not the case with Abcalis® Multiclonals™.

As shown on the right, our Multiclonal™ anti-human antibody is even capable of showing higher binding capacity than the same amount of animal derived polyclonal antibody, possibly due to the fact that every ant body in the Multiclonal™ mix is highly specific only for the target antigen. This can of course never be the case in polyclonal sera.

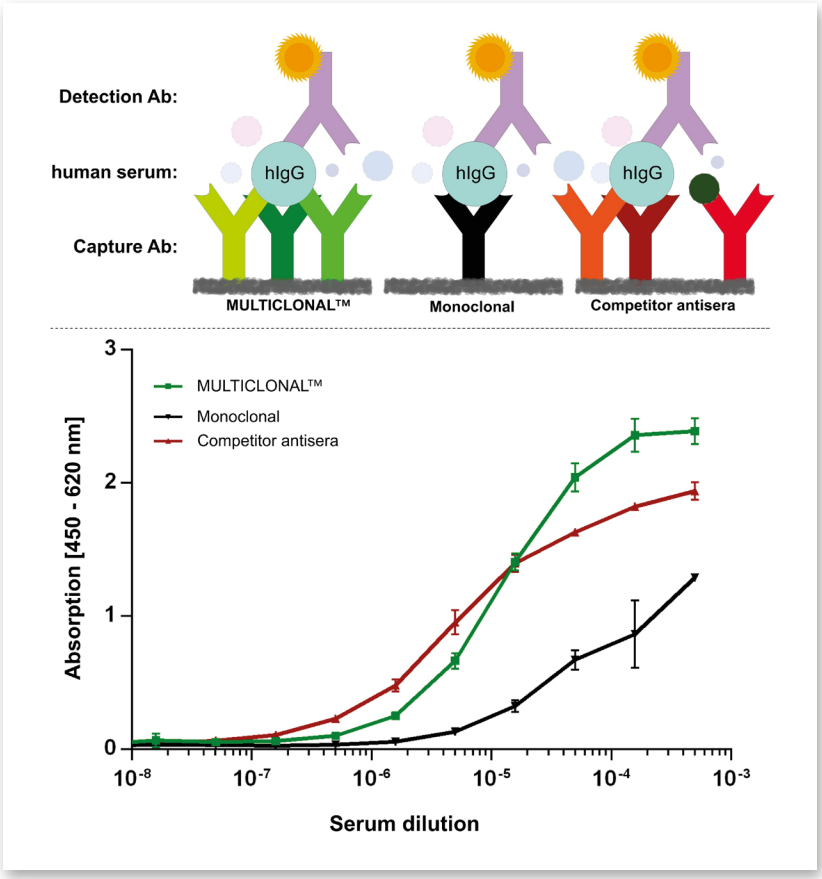

Fig. 3: Human IgG detection in serum sample on plate immobilized anti-human Multiclonal™, monoclonal, and polyclonal antibody.

Improved sensitivity of Multiclonal™ antibodies

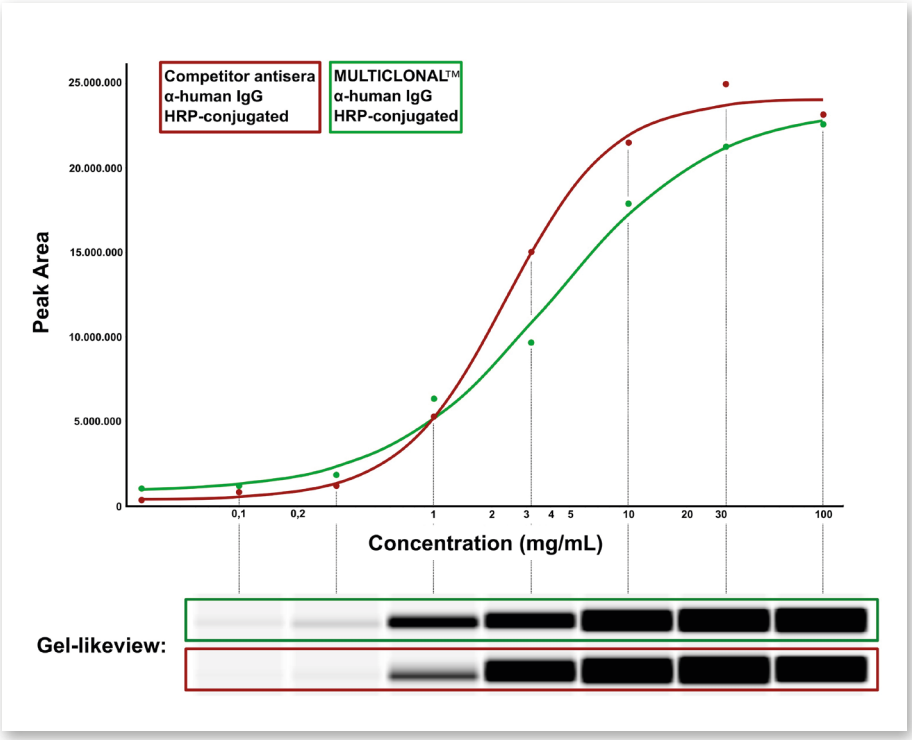

Fig. 4: Quantitative immunodetection of antigen bound human IgG with the automated protein Simple Western Immunoassay™ system using either Multiclonal-HRP or HRP labeled goat antiserum.

Simple Western Immunoassay[2] is an automated gel free and blot-free method to provide quantitative immunoblot results. An antigen target was separated by capillary electrophoresis according to its size and then identified by antigen specific primary human IgG. Either Abcalis® Multiclonal™ or animal derived secondary antibody anti-human IgG, both conjugated with HRP, were used to detect and visualize the reaction with a chemiluminescent substrate. The resulting signal allows quantification over a large range of concentrations (Fig. 4).

Abcalis® Multiclonal™ anti-human IgG HRP showed comparable binding and higher dynamic range compared to a typical animal based secondary antibody.

[2] Simple Western Immunoassay™ and Protein Simple™ are trademarks of ProteinSimple, San Jose, USA

About Abcalis®

Abcalis® is a young biotechnology company explicitly founded for the development, production and distribution of completely animal free recombinant antibodies. We want to help pave the way to a more sustainable future in antibody-based diagnostics and research and to simultaneously end the suffering of millions of animals each year.

Our products provide a whole new category of solutions to replace all of the current types of the products on the research and diagnostics antibody market. These types include:

- animal derived serum products (polyclonals)
- animal derived monoclonals (hybridoma derived antibodies)
- recombinant antibodies which are derived from animal immunizations

All of our antibodies are made completely *in vitro* without immunizations through a technology one of our founders co-invented 30 years ago: Antibody Phage Display. At Abcalis, we strive to advance and improve the technology even further. This already enabled us to complete an array of successful industry projects and collaborations with our customers and we are also strongly committed to continue extending our future catalog product portfolio. Additionally, we are also implementing efforts to replace every animal derived material in the entire process, e.g. in cultivation media or blocking substances. This defines our Why: Because we want everyone to benefit from truly vegan antibodies.

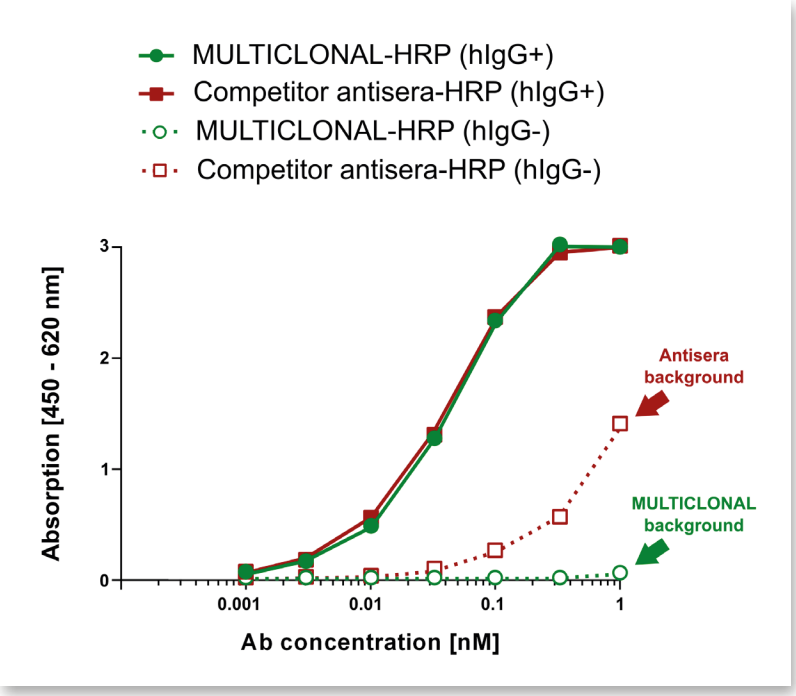

Fig. 5: Anti-human Multiclonal-HRP vs. competitor goat antisera-HRP detection of antigen bound hIgG in microtiter plate.

Multiclonals™ anti-human IgG shows lower cross-reactivity

Antigen bound primary hIgG antibody was detected via HRP-conjugated anti-human secondary antbiodies in ELISA.
Direct comparison of Abcalis® Multiclonals™ with typical animal derived secondary antibodies (Fig. 5) revealed a considerably lower cross reactivity binding by our recombinant product.

In animal sera, the presence of Immunoglobulins with unknown specificities can lead to unwanted reactivities, as in the presented case.

Abcalis® Multiclonals™ are therefore the right choice when an assay with high specificity and low background is required.

Multiclonals™ anti-human IgG bind all subclasses

Abcalis® anti-human IgG Multiclonals™ are carefully adjusted antibody mixtures able to recognize different epitopes on all four different subclasses of human IgG (Fig. 6).

This provides the classical multi-epitope recognition which is typical for polyclonal antisera.

Adjusted IgG1 binding mixes with different specificities are also possible and can be provided by Abcalis®.

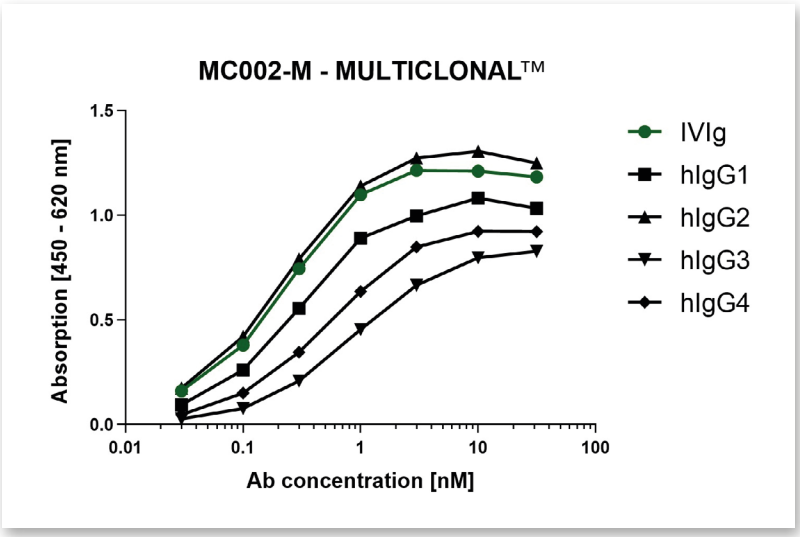

Fig. 6: Multiclonals™ binding to plate immobilized hIgG subclasses.

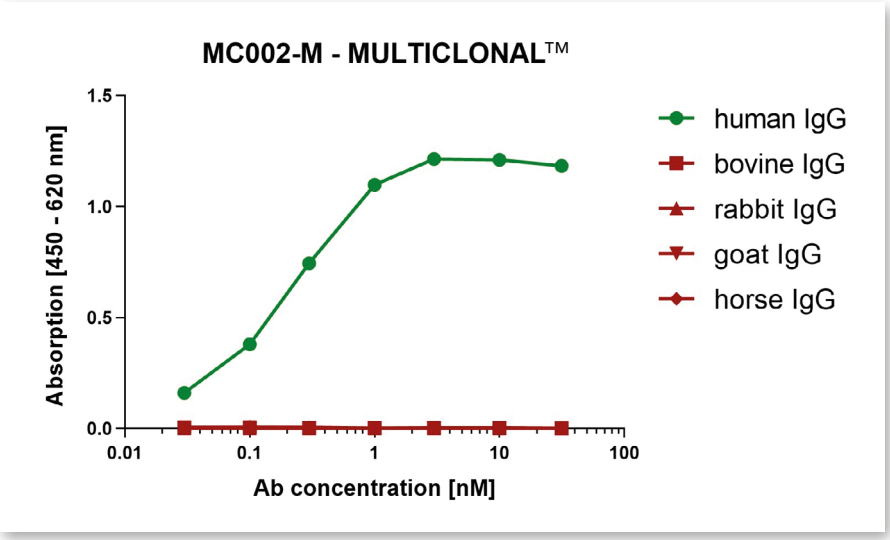

Fig. 7: Multiclonal™ anti-human IgG binding to immobilized IgG molecules from different species.

Multiclonals™ have a predesigned cross-reactivity profile

Abcalis® Multiclonal™ anti-human IgG shows specific binding to human IgG (Fig. 7).
Each individual antibody of an anti-human IgG Multiclonal™ mix is selected for its absence of cross-reactivity towards Immunoglobulins of other species. The absence of species cross-reactivity is not the result of laborious and costly cross-adsorption, but the effect of a negative selection and competition process already included during phage display panning on antigens. However, if a specific cross-reactivity is needed for an assay, Abcalis® Multiclonals™ are also adjustable to that.

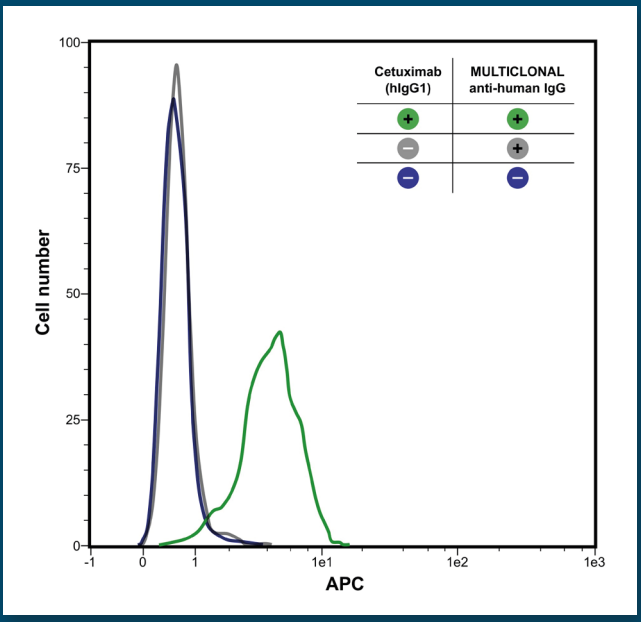

Fig. 8: Multiclonals™ detection of recombinant hIgG1 Cetuximab antibody binding on EGFR-positive cells.

Multiclonals™ also work perfectly in flow cytometry

Cell lines express >1000 proteins on their surface, making specificity testing of antibodies on cells highly probative. EGFR-positive EXPI cells were stained with Cetuximab hIgG1 antibody against EGFR.

Cetuximab detection with secondary anti-human Multiclonal™ (Fig. 8) is highly specific, as shown by the complete absence of background staining on Cetuximab unstained cells.

Multiclonals™ are highly pure reagents

Antibodies composing Multiclonals™ anti-human IgG mix are individually tested in coomassie brilliant blue stained SDS-PAGE (Fig. 9-A) and size exclusion chromatography (Fig. 9-B) to exclude the presence of impurities and aggregates formation.
Molecular weight distribution is also assessed after log term storage and protein stability stress test.

Abcalis® Multiclonals™ mix remain monomeric and stable over time despite thermal and physical acute stresses.

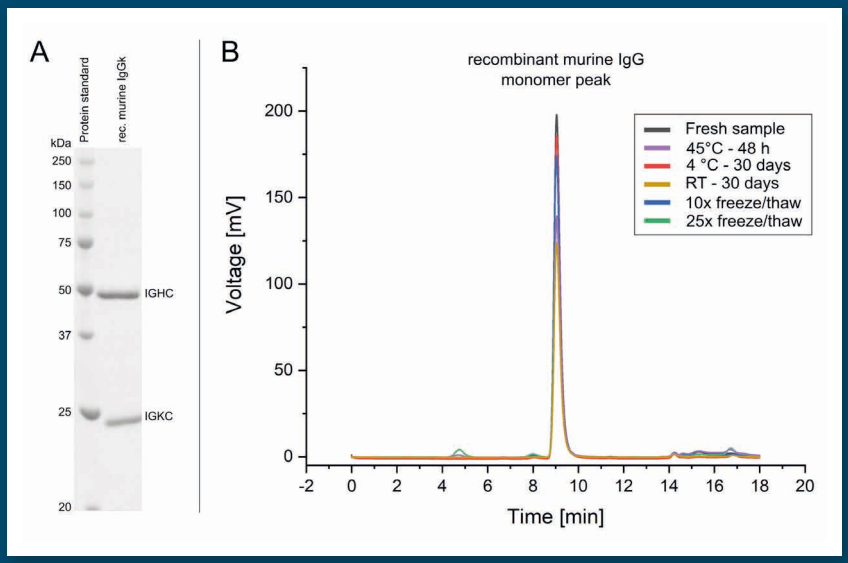

Fig. 9: Gel staining of reduced recombinant murine IgG (left). Size exclusion chromatography of a representative murine recombinant IgG component of the Multiclonal™ anti-human IgG on a Superdex 200 after storage and stress test.

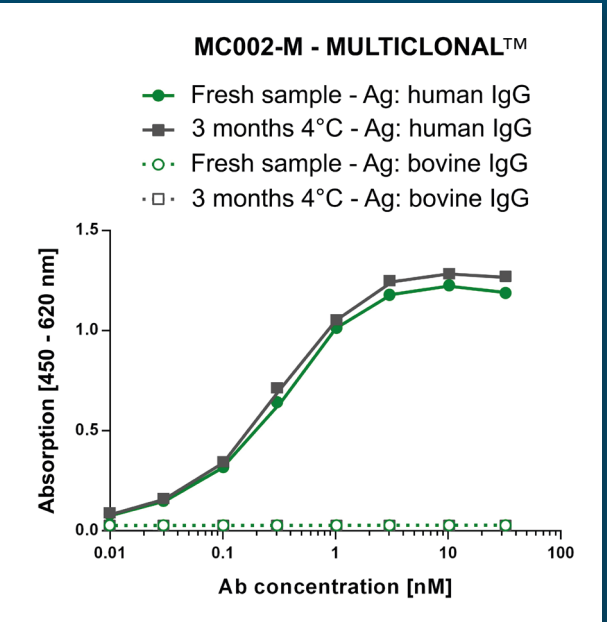

Fig. 10: Multiclonals™ binding to plate immobilized hIgG subclasses.

Multiclonals™ are robust reagents

Abcalis® Anti-human IgG Multiclonals™ can, even in the absence of additional stabilizers, be stored for several months at 4°C without risk of harming its binding activity (Fig. 10).

This is possible since each individual antibody in the mix has been carefully selected not only for high specificity and lowest background binding, but also for high stability and long shelf life. In fact, each antibody is tested to tolerate 45°C, freeze-drying process induced stress,
and >25 freeze/thaw cycles (Fig. 9).

Abcalis Animal Use Statement

Abcalis® has the vision to generate and produce antibodies entirely without animal experiments.

We achieve this by using the animal free method of *in vitro* selection by phage display from antibody gene libraries to generate monoclonal antibodies. Abcalis® does not generate hybridomas.

Moreover, in the production process, our cultivation media are free of animal derived materials, like fetal calf serum, BSA or other animal derived materials.
Abcalis® antibodies may contain animal derived sequences. Examples are the genes encoding the mouse Fc, rabbit Fc or other animal Fc parts of Abcalis® antibodies. The use of these constant region sequences is unavoidable to provide compatibility to our customers' applications. Abcalis® did not use any laboratory animals to obtain these sequences, which were chemically synthesized based on publications or obtained as recombinant DNA from commercial sources.

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