MULTICLONALS
Anti-human IgG secondary antibodies

An animal free recombinant antibody mix for unlimited reproducibilty

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. The generation and production of MULTICLONALS is entirely animal free.

MULTICLONALS can replace and surpass animal antisera

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.

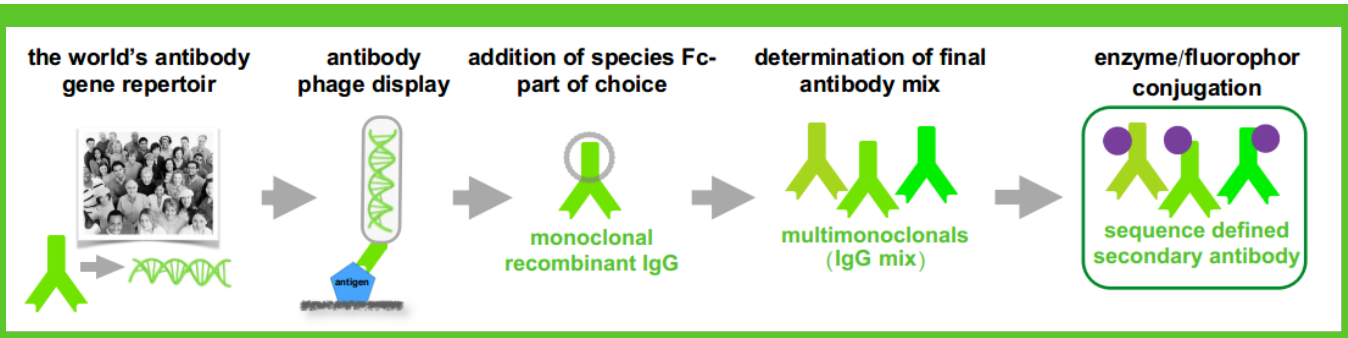

Figure 1: How MULTICLONALS are generated by phage display.

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.

Advantages of MULTICLONALS for your work

First, Abcalis® secondary antibodies (Fig. 2) are only target specific antibodies. They do not contain 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 translates to superior specificity and offers the possibility for 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 a critical aspect in respect of high QC standards and IVD regulations.

Third, Abcalis® antibodies are made entirely *in vitro* by recombinant methods and are therefore animal free. They come as highly purified Protein A protein. 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.

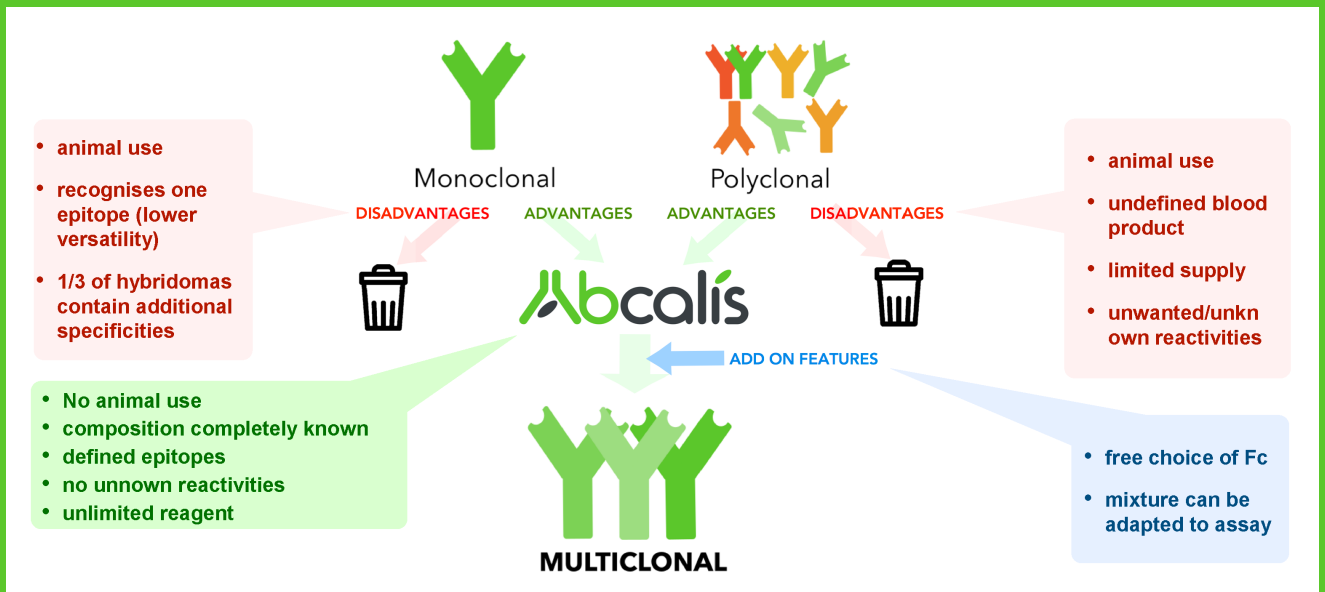

Figure 2: Get the best of three worlds: MULTICLONALS combine the best of polyclonal antisera and hybridoma monoclonal antibodies, while eliminating their disadvantages and adding the advantages of completely defined recombinant reagents.

[1] Bradbury et al. (2018). When monoclonal antibodies are not monospecific: Hybridomas frequently express additional functional variable regions. MAbs 10(4):539-546

MULTICLONALS capture human IgG from serum samples

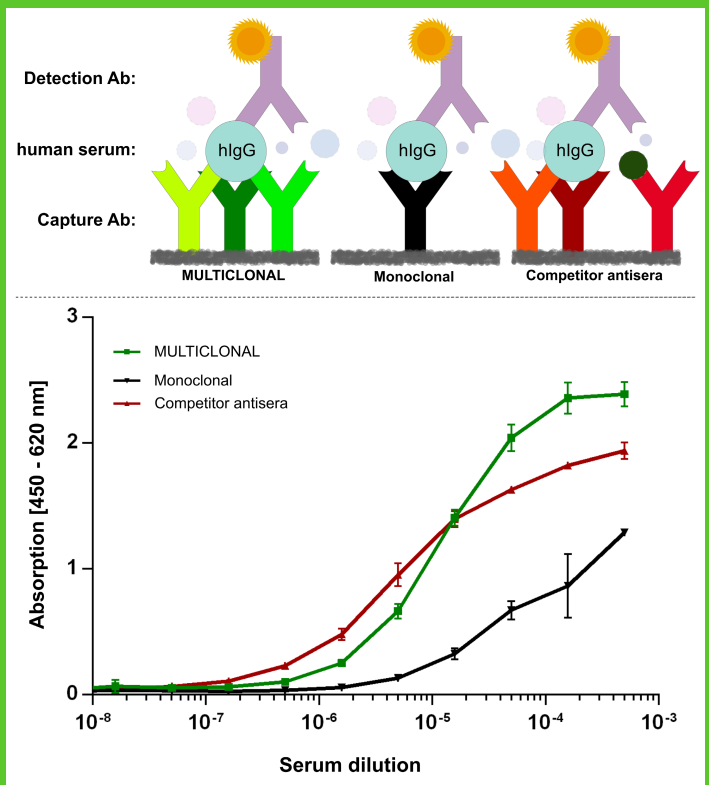

Figure 3: human IgG detection in serum sample on plate immobilized anti-human MULTICLONAL, monoclonal, or polyclonal antibody.

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.

MULTICLONAL anti-human antibody even shows higher binding capacity than the same amount of animal derived polyclonal antibody, possible due to the fact that every antibody in the MULTICLONAL mix is specific for the antigen while this is never the case in polyclonal serum.

Improved sensitivity of MULTICLONAL antibodies

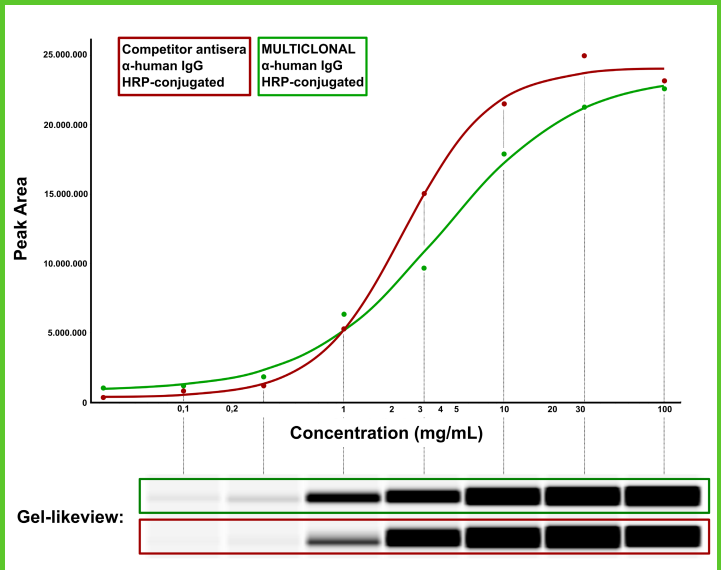

Figure 4: Quantitative immunodetection of antigen bound hIgG with the automated Protein Simple Western Immuno-assay™ system using either MULTICLONAL-HRP or HRP labeled goat antiserum.

Simple Western Immunoassay[2] is an automated gel free and blot–free method to provide quantitative immuno-blot results. An antigen target was separated by capillary electrophoresis according to its size, then identified by antigen specific primary human IgG. Either Abcalis® MULTICLONAL or animal derived secondary antibody anti-human IgG 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

MULTICLONAL anti-human IgG shows lower cross reactivity

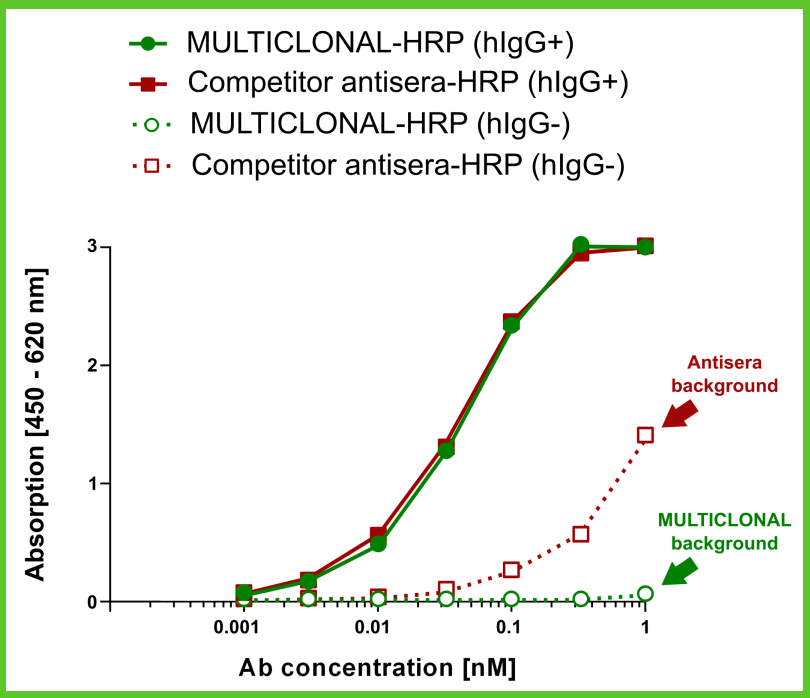

Figure 5: anti-human MULTICLONAL-HRP vs. competitor goat antisera-HRP detection of antigen bound hIgG in microtiter plate.

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 of the recombinant product.

In animal sera, the presence of Immunoglobulins with unknown specificities can lead to unwanted reactivity, 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 subclassess

Abcalis® anti-human IgG MULTICLONALS consist of carefully adjusted Ab 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®.

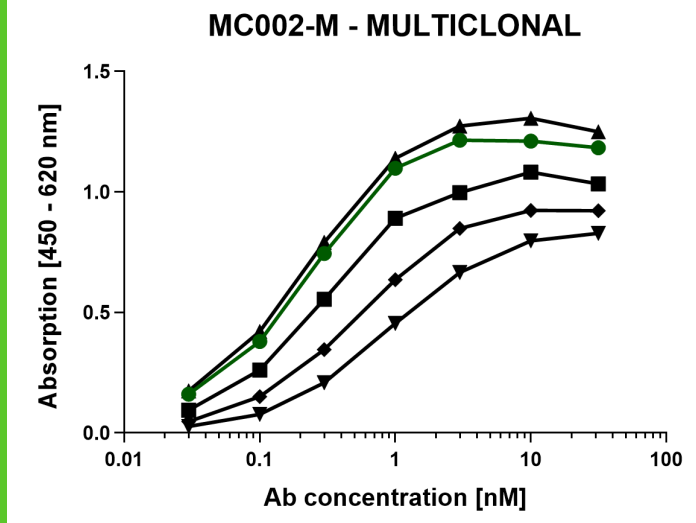

Figure 6: MULTICLONALS binding to plate immobilized hIgG subclasses. MULTICLONALS epitope diversity guarantees comparable detection to each hIgG subclass.

MULTICLONALS have a predesigned cross- reativity profile

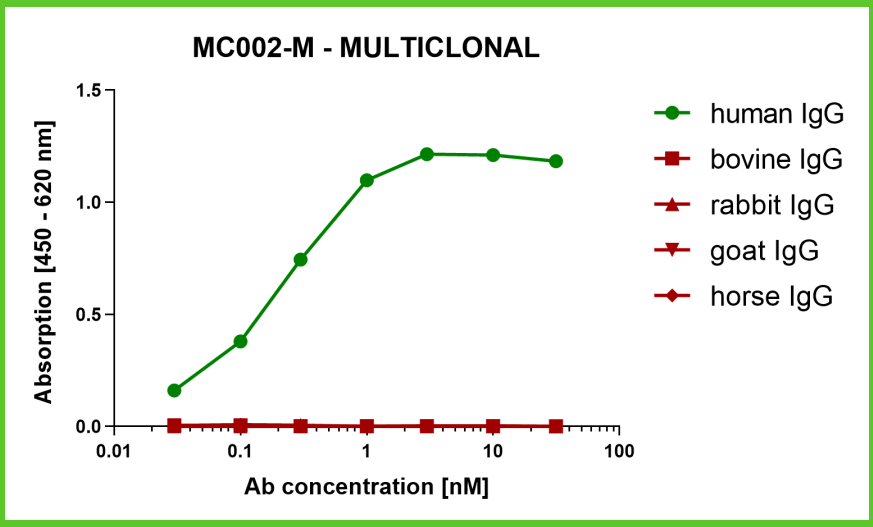

Figure 7: MULTICLONAL anti-human IgG binding to immobilized IgG molecules from different species.

MULTICLONAL anti-human IgG shows specific binding to human IgG (Fig. 7).

Each individual antibody of an Abcalis® 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 incuded in their very making during phage display panning on antigens. However, if a specific cross-reactivity is needed for an assay, Abcalis® MULTICLONALS are also adjustable to that.

MULTICLONALS also work perfectly in flow cytometry

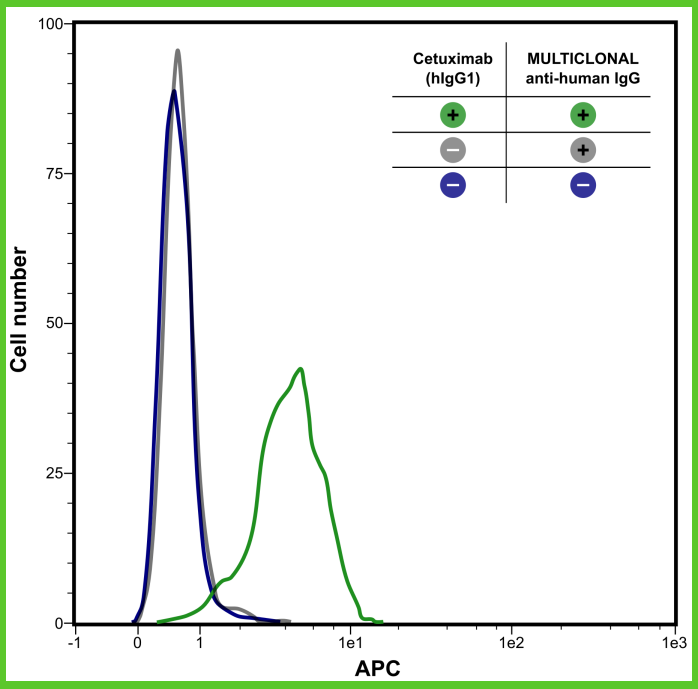

Figure 8: MULTICLONALS detection of recombinant hIgG1 Cetuximab Ab binding on EGFR-positive cells.

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 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. 9).

This is possible since each individual antibody in the mix has been carefully selected not only for high specificity/ lowest background binding but also already 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. 10).

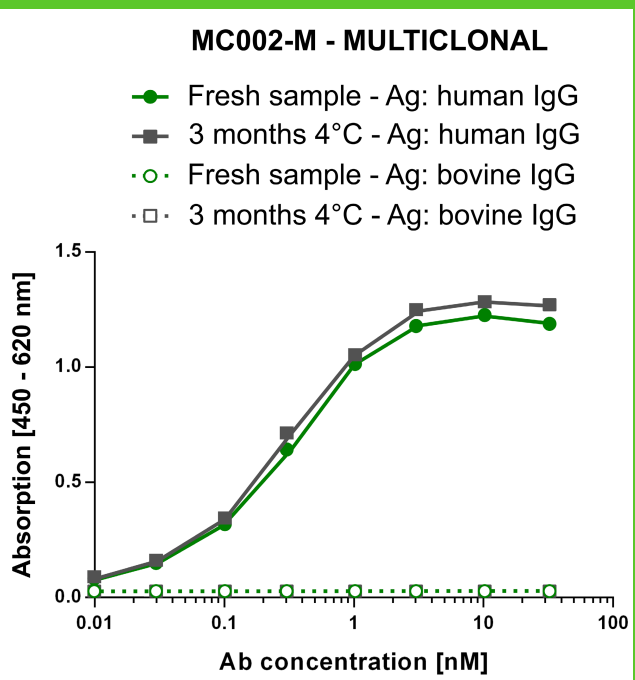

Figure 9: Anti-human IgG MULTICLONAL binding in ELISA to immobilized hIgG or CTR- antigen, before and after storage in 1xPBS at 4°C for several months.

MULTICLONALS are highly pure reagents

Antibodies composing MULTICLONAL anti-human IgG mix are individully tested in coomassie brilliant blue stained SDS-PAGE (Fig. 10-A) and size exclusion chromatography (Fig. 10-B) to exclude the presence of impurities and aggregates formation.

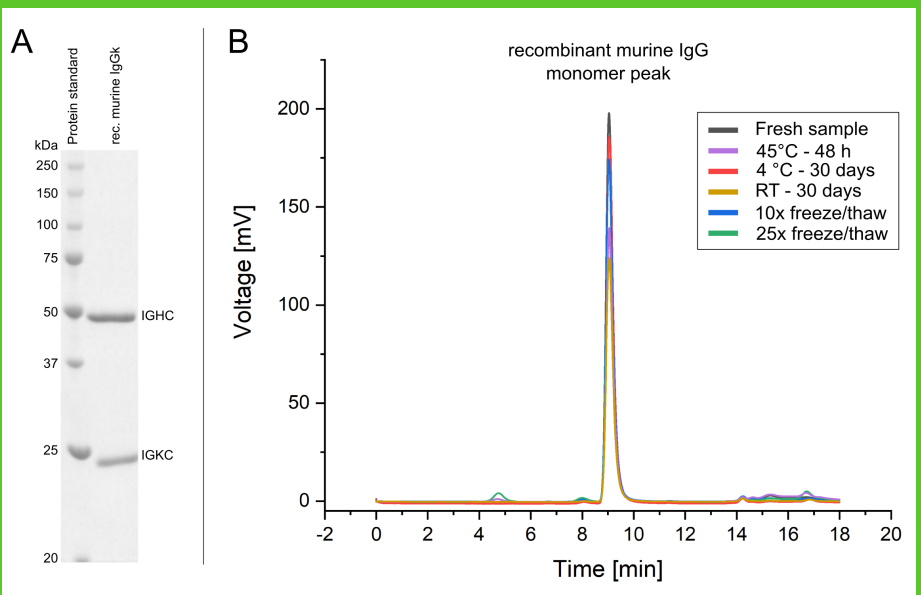

Figure 10: Gel staining of reduced recombinant murine IgG (left). Size exclusion chromatography of a representative murine recombinant IgG component of the MULTICLONALS anti-human IgG on a Superdex 200 after storage and stress test.

Molecular weight distribution is also assessed after log term storage and protein stability stress test.

Abcalis® MULTICLONAL mix composing antibodies remain monomeric and stable over time despite thermal and physical acute stresses.

About Abcalis:

All Abcalis® Multiclonals are produced recombinantly and can therefore benefit from the established advantages of polyclonal mixtures, while eliminating the downsides regarding long-term availability and reproducibility, antigen purification and lack of continuous product identity. Our antibodies are selected by 2018 Nobel-Prize Winning technology Antibody Phage Display *in vitro*. Our process is based on know-how and libraries developed by one of its inventors, building on the experience of >30 years.

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 or 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.