

Frequently Asked Questions (FAQs): Antibodies

General

Should I choose mAb or pAb?

The production of antibodies involves the immunisation of one or more animals with an antigen in order to elicit an immune response within the host animal. A number of key factors will determine the choice between polyclonal or monoclonal antibodies.

	Polyclonal Antibodies (pAbs)	Monoclonal Antibodies (mAbs)
Production	Host animals are immunised with a predefined antigen, causing an immune response resulting in the secretion of antibodies by different B-lymphocytes. As each B-lymphocyte recognises a different epitope, this process results in a heterologous population of antibodies which can then be recovered directly from the host's serum.	A monoclonal hybridoma cell line is first created by the fusion of antibody-secreting immune cells from immunised mice, with immortal myeloma cells. The resultant hybridoma cell line then expresses a specific antibody when cultured <i>in vitro</i> . The antibodies may subsequently be purified from the cell culture supernatant, if required.
Timescale	Standard schedules are 77-98 days in rabbit.	3-9 months, due to the time it takes to develop a successful hybridoma cell line with first screen data typically available at 3 months.
Specificity	Broad spectrum recognition across the target due to the presence of a cocktail of antibodies that recognise multiple epitopes.	Highly specific antibodies. mAbs recognise a single epitope on a single antigen as long as it is unique.
Advantages	pAbs provide more robust detection due to the presence of multiple epitopes.	A renewable resource due to the creation of the hybridoma cell line that permits unlimited production of mAbs.
Batch-to-batch variability	Batch-to-batch variation is likely, due to the inherent variability in the immune response of the host animals.	Low batch-to-batch variation due to the clonal population of cells and single antibody.
Applications	Western blot, ELISA development, affinity purification, IP/IHC. Supply of antibody is limited by the available harvest and terminal bleeds.	Western blot, ELISA development, IP/IHC, affinity purification, flow cytometry, infectious disease characterisation, potential therapeutics, target validation and diagnostic tool.

How much antigen do I need to supply?

Typical (minimum) recommended antigen concentrations per immunisation are as follows:

Host Species	Minimum amount of antigen required	Deliverable: Average serum yield per animal
Mouse	TOTAL: 1,000 µg (1 mg for 4 animals)	0.3 - 0.5 ml
Rat	TOTAL: 1,000 µg (1 mg for 4 animals)	2 - 3 ml
Rabbit	200 µg (0.2 mg) of antigen (0.4 mg/mL) for each of 6 immunisations. TOTAL: 1,200 µg (2,400 µg for 2 animals)	50-100 ml depending on breed
Sheep / Goat	200 - 500 µg (0.2 - 0.5 mg) of antigen (0.4 - 1 mg/ml dependent on antigenicity) for each of 6 immunisations. TOTAL: 1,200 - 3000 µg	750 – 1,000 ml with harvest bleeds also available to increase yield
Alpaca / Llama	200 - 500 µg (0.2 - 0.5 mg) of antigen (0.4 - 1 mg/ml dependent on antigenicity) for each of 6 immunisations. TOTAL: 1,200 - 3000 µg	500 – 750 ml with harvest bleeds also available to increase yield

Please note: Due to natural variations, exact volumes cannot be guaranteed

What is the purity of custom antibodies?

Various purification options offering different levels of purity are available and we would be happy to discuss these on a project-by-project basis with affinity purification being the gold standard for polyclonal antisera.

How much purified antibody or antisera will I receive for custom antibodies?

The concentration of purified antibody available at the end of the project will depend upon many factors, including the method of purification chosen. If a specific amount of purified antibody is required, this should be indicated at the commencement of the project to ensure that this is taken into account at the project design stage. Typically, working concentrations need to be determined empirically.

How should I store my antibody?

Custom antibodies typically should be considered to have a shelf life of at least 1 year. However aliquots may be frozen at -20°C or below to increase the shelf life. Preservatives may be added, as required, in order to prevent microbial contamination.

What specifications are provided for antibodies?

The following parameters will be reports on a Certificate of Analysis for each antibody:

- Concentration
- Label
- Buffer
- Preservative
- Volume

What purification methods are available for custom antibodies?

Purification of IgG is typically carried out by Protein A or Protein G affinity purification. However, APS offers a range of purification methods for monoclonal and polyclonal antibodies. The purification methods used are dependent upon the isotype of the antibody for culture (IgA, IgG or IgM).

Other purification methods available include:

- Ammonium Sulphate Precipitation
- Dialysis & Ultrafiltration
- Ion Exchange Chromatography
- IgM Purification – size exclusion
- IgY Purification – salt fractionation

What other antibody processing and modification options are available?

- SDS-PAGE analysis (H and L chain composition)
- Antibody Fragmentation for generation of fragments including Fab, F(ab')₂ and Fc
- Preparation of affinity matrices using customer-specific immobilised peptide and protein ligands and antibodies
- Metal chelate affinity chromatography

What additional testing is available?

- Endotoxin testing
- Mycoplasma testing
- Labelling – HRP, Biotinylation, fluorochromes, etc ELISA analysis of serum bleeds
- Immune cell/spleen preparations for mAb production

What is the recommended storage and handling for antibodies?

Antibodies are relatively fragile so repeated freeze/thaw cycles should be avoided. If an antimicrobial agent such as sodium azide is added, product may be stored at +4°C for up to 2 weeks. For long term storage, it is recommended to aliquot the antibody and store at -20°C.

For FITC labelled rabbit clonal antibodies the storage conditions are different. We recommend storing undiluted at +4°C. Do not freeze. Protect from prolonged exposure to light.

How are antibodies shipped?

Antibodies are supplied as frozen material and will be shipped at -20°C.

For additional materials supplied as part of a custom antibody product service, shipping conditions are as follows:

- Test bleeds will be supplied as serum and shipped frozen on dry ice.
- Antisera will be supplied as serum and shipped frozen on dry ice.

- Custom cell lines will be shipped frozen on dry ice..
- Custom peptides are supplied as lyophilised material and shipped at ambient temperature, usually with a desiccant.

Custom Polyclonal Antibodies

What species are available?

Mouse, rat, Rabbit, Goat, Sheep, Alpaca, Llama, Chicken (blood and eggs) and Emu (eggs).

What is the typical project length?

A typical polyclonal project protocol is a 77 day protocol. However, protocols may be adapted to suit the antigen, the antigenic response to the antigen and titre of antibody required.

Custom Monoclonal Antibodies

What species are used in the production of mAbs?

We routinely use Mouse Balb/c as the host species for the production of mAbs although other strains of mice are available.

What is the typical project length?

A project can take from 3 to 9 months depending on the immunisation schedule and overall project complexity. All projects are individually designed to offer the best possible outcome within the constraints of budget.

Rabbit Clonal Antibodies

What is the difference between monoclonal and monospecific clonal antibodies?

Clonal antibodies are monospecific such as monoclonals. The principal difference between these two types of antibodies is that the DB Biotech clonal antibodies recognize solely very specifically selected linear epitope on the antigen molecule after its detailed proteomic analysis. In contrast, the monoclonal antibodies recognize very often steric epitopes that frequently change their conformation during tissue preparation, protein extraction, etc., making the corresponding monoclonal antibody unspecific, less avid and in extreme cases, non-functional.

What is the advantage of using rabbit clonal antibody produced from crude rabbit antiserum by in vitro cloning technology, when compared to monoclonal antibodies from hybridoma fusion cell lines?

Outside of strictly specific linear epitopes characterisation, clonal antibodies are mature immunoglobulins (IgG), with the complete post translational modification of IgG molecule, mainly glycosylation, which plays an important role in stability of immunoglobulins. There are many reports in the literature describing the incomplete glycosylation patterns of monoclonal antibodies produced from hybridoma cell lines, which has an important influence on stability, affinity and avidity of antigen/antibody interactions.

Why are there different clones of the same antibody for the application in research (western blot, immunoprecipitation, ELISA) and clinical diagnostic (IHC-P)?

When designing the clonal antibody for IHC-P, the severe and irreversible denaturation of the antigen including the conformation of the corresponding epitope against which the clonal antibody is produced is taken into consideration. In most cases, the epitope for this application will be selected on different conformational criteria than for the western blot or ELISA application, where the original structure of the antigen is preserved or where the denaturation is considerably weaker. This is the reason why for most cases, different epitopes are designed for various applications and consequently different clonal antibodies are produced characterizing various epitopal sequences of the target protein molecule.

Why are clonal antibodies produced by original in vitro cloning technology exceptional?

Clonal antibodies are exceptional because they recognize their target (epitope) with the highest affinity, avidity and specificity that remains unchanged even after the important changes in conformation of the antigen – such as denaturation during the fixation of the tissue, protein transfer in the case of western blot-related procedures, or as a consequence of the protein-protein interactions.

Are clonal antibodies similar to immunoaffinity purified polyclonals?

No. Immunoaffinity purified polyclonal antibodies (whether the immunogen is the whole protein or selected peptide) are always represented by numerous fractions of immunoglobulins corresponding to all epitopes in the immunogenic sequence. Rabbit clonal antibody is a homogenous fraction of immunoglobulin (IgG) corresponding exclusively to one and only specifically defined epitope on the antigen molecule.

Support

Antibody Production Services is a division of Life Science Group Ltd.

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