Antibodies

WHITE PAPER

LIFE SCIENCE RESEARCH

How to choose an antibody?

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Make an enlightened decision when selecting your antibody.

Over the years, antibodies have clearly become indispensable tools for biological research applications. The unique specificity of antibodies for a wide variety of types of molecules has generated numerous antibody-based methods, from the tried and true western-blot to fully automated multiplex single molecule immunoassay systems. Successful immunoassays are dependent on the researcher's ability to identify, select or produce the best antibody in an application-specific manner.

The key and lock image often used to describe the selectivity of an antibody to its antigen can also be applied to the selection of an antibody to achieve a project's goal : each project is unique and calls for particular features of the antibody.

This white paper is a guide offering suggestions to research and clinical laboratories on how to choose an antibody. Catalogue or custom, monoclonal or polyclonal, pre-coupled or unmodified, how to select the best host, against which antigen type, we'll show you how to decide.

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CATALOGUE VS CUSTOM ANTIBODIES

Catalogue antibody, the rapid choice

Catalogue antibodies are research reagents that are directly available from a supplier for the detection of a particular antigen (Table 1).

The number of catalogue antibodies rises continually. Some companies offer hundreds of thousands of antibodies while other companies prefer to focus on specific themes such as disease areas (e.g. apoptosis), application (e.g. cell and tissue imaging) or types of antibodies (e.g. secondary antibodies). Catalogue antibodies are often commercialised when there is a significant scientific interest in a particular antigen (e.g. clone 33D3 to 5-methyl-cytosine, a monoclonal antibody which targets the modified DNA base 5-methylcytosine).

When searching through the antibody catalogue it is important to pay attention to the information provided about the antibody as this will have a direct impact on the suitability of the antibody in a particular application : is it a polyclonal or monoclonal antibody? From which species is the antiserum? Is it purified and how (e.g. affinity, protein A/G)? Is it furnished with additives (e.g. sodium azide, BSA, glycerol)? But also information regarding the target antigen : which antigen was used to raise the antibody (e.g. hapten, peptide on carrier, protein, modified protein) ? How structured was the antigen used for generating the antiserum (e.g. denatured protein, native protein)? Has the antibody been validated for a particular immunoassay and antigen type? If details are missing for example for "proprietary" reasons, scientists should be aware that they are missing crucial information necessary for making an informed decision.

Catalogue antibodies are directly available and less expensive, but they can't be personalised in contrast to custom antibodies.

Custom antibody, a solution to meet specific needs

Custom antibodies are client specific; they involve the generation of a specific antibody using a defined and suitable host against a defined target antigen to generate the necessary quantities of antibody for the application of the customer.

Given the large number of options involved in the generation of a custom antibody, and the importance of each option, it is critical for the supplier to understand the goals and experimental conditions of the planned immunoassay in order to best customise the antibody programme.

Below we outline the impact of each choice in order to help you make the best choice for your application.

Cat #	Product Description	Clone	Size
MMS-101P-XXX	*Optim*Ab™ HA.11, mAb, purified	16B12	100µg, 500µg
AFC-101P-500	*Optim*Ab™ HA.11, mAb, Affinity Matrix	16B12	500µg
BIOT-101L-050	*Optim*Ab™ HA.11, mAb, Biotin Labeled	16B12	50µg
FITC-101L-050	*Optim*Ab™ HA.11, mAb, FITC Labeled	16B12	50µg
PRB-101P-100	*Optim*Ab™ HA.11, pAb, purified	N/A	100µg
SIG-39320-XXX	*Optim*Ab™ Beta-Amyloid 1-16, mAb, purified	6E10	100µg, 500µg
SIG-39340-100	*Optim*Ab™ Beta-Amyloid 1-16, mAb, Biotin Labeled	6E10	100µg
SIG-39345-050	*Optim*Ab™ Beta Amyloid 1-16, mAb, HRP Labeled	6E10	100µg
SIG-39220-XXX	*Optim*Ab™ Beta-Amyloid 17-24, mAb, purified	4G8	100µg, 500µg
SIG-39240-100	*Optim*Ab™ Beta-Amyloid 17-24, mAb, Biotin Labeled	4G8	100µg
SIG-39245-100	*Optim*Ab™ Beta Amyloid 17-24, mAb, HRP Labeled	4G8	100µg
SIG-39142-050	*Optim*Ab™ Beta-Amyloid 1-42, mAb, purified	12F4	50µg
SIG-39142-250	*Optim*Ab™ Beta-Amyloid 1-42, mAb, purified	12F4	250µg
SIG-39144-050	*Optim*Ab™ Beta-Amyloid 1-42, mAb, Biotin Labeled	12F4	50µg
SIG-39147-050	*Optim*Ab™ Beta-Amyloid 1-42, mAb, HRP Labeled	12F4	50µg
MMS-435P-XXX	*Optim*Ab™ Neuronal Class III beta-Tubulin, mAb, purified	TUJ1	100µg, 200µg
PRB-435P-050	*Optim*Ab™ Neuronal Class III beta-Tubulin, pAb, purified	N/A	50µg
PRB-278P-100	*Optim*Ab™ Pax-6, pAb, purified	N/A	100µg
SIG-39138-050	*Optim*Ab™ sAPPbeta, pAb, purified	N/A	50µg
SMI-312P-050	*Optim*Ab™ Pan-Axonal Neurofilament Marker, mAb, purified	SMI-312	50µg
SMI-310P-050	*Optim*Ab™ Neurofilament H&M Phosphorylated, mAb, purified	SMI-310	50µg
SMI-32P-050	*Optim*Ab™ Neurofilament H Non-Phosphorylated, mAb, purified	SMI-32	50µg
SMI-34P-050	*Optim*Ab™ Neurofilament H Phosphorylated, mAb, purified	SMI-34	50µg
SMI-31P-050	*Optim*Ab™ Neurofilaments, Phosphorylated, mAb, purified	SMI-31	50µg
SMI-35P-050	*Optim*Ab™ Neurofilaments, Hypophosphorylated, mAb, purified	SMI-35	50µg
MMS-126P-050	*Optim*Ab™ RNA Polymerase II, mAb, purified	8WG16	50µg
MMS-128P-050	*Optim*Ab™ RNA Polymerase II, mAb, purified	CTD4H8	50µg
MMS-164P-050	*Optim*Ab™ c-Myc, mAb, purified	9E11	50µg
MMS-150P-050	*Optim*Ab™ c-Myc, mAb, purified	9E10	50µg
BIOT-150L-050	*Optim*Ab™ c-Myc, mAb, Biotin labeled	9E10	50µg
SIG-39840-050	*Optim*Ab™ LRRK2, mAb, purified	MC.028.83. 76.242	50µg
SIG-39725-100	*Optim*Ab™ alpha-Synuclein, mAb, purified	LB509	100µg
MMS-5085-050	*Optim*Ab™ alpha-Synuclein, mAb, purified	Syn303	50µg
SIG-39730-100	*Optim*Ab™ alpha-Synuclein, mAb, purified	4B12	100µg
SIG-39720-100	*Optim*Ab™ alpha-Synuclein, mAb, purified	4D6	100µg
SIG-39620-100	*Optim*Ab™ Prion, mAb, purified	3F4	100µg
SIG-39810-100	*Optim*Ab™ Prion, mAb, purified	6D11	100µg
MMS-5018-050	*Optim*Ab™ Tau, mAb, purified	77E9	50µg
SIG-39413-050	*Optim*Ab™ Tau, mAb, purified	Tau 5	50µg
SMI-99P-100	*Optim*Ab™ Myelin Basic Protein, mAb, purified	SMI 99	100µg
PRB-145P-050	*Optim*Ab™ Loricrin, pAb, purified	N/A	50µg
PRB-440P-050	*Optim*Ab™ Nonmuscle Myosin Heavy Chain II-A, pAb, purified	N/A	50µg
PRB-445P-050	*Optim*Ab™ Nonmuscle Myosin Heavy Chain II-B, pAb, purified	N/A	50µg
MMS-257S-050	*Optim*Ab™ Ubiquitin, mAb, purified	PAD1	50µg
SIG-38710-100	*Optim*Ab™ P-Glycoprotein 3 (MDR3), mAb, purified	C219	100µL
MMS-106P-100	*Optim*Ab™ Cre Recombinase, mAb, purified	7.23	100µg
BIOT-106L-050	*Optim*Ab™ Cre Recombinase, mAb, Biotin Labeled	7.23	50µg
PRB-106P-100	*Optim*Ab™ Cre Recombinase, pAb, purified	N/A	100µg
MMS-570S-050	*Optim*Ab™ Nestin, mAb, purified	10C2	50µg
MMS-120P-100	*Optim*Ab™ Nuclear Pore Complex Proteins, mAb, purified	MAb414	100µg
PRB-417P-050	*Optim*Ab™ Filaggrin, pAb, purified	N/A	50µg
SIG-3730-250	*Optim*Ab™ Lymphatic Endothelial Marker, mAb, purified	D2-40	250µL
SIG-3611-250	*Optim*Ab™ GCDFP-15, mAb, purified	D6	250µL
MMS-162P-100	*Optim*Ab™ Human Cytokeratin-8, mAb, purified	1E8	100µg
SIG-3465-250	*Optim*Ab™ Cytokeratin, mAb, purified	OSCAR	250µL
MMS-159S-100	*Optim*Ab™ Cytokeratin-10, mAb	DE-K10	100µL
MMS-130P-100	*Optim*Ab™ AU1 Epitope Tag, mAb, purified	AU1	100µg
PRB-276P-100	*Optim*Ab™ Pax-2, pAb, purified	N/A	100µg
BI-MECY-XXXX	*Optim*Ab™ 5-Methylcytosine, mAb	33D3	100µg, 500µg, 1mg

Table 1. Example of catalogue antibodies

ABOUT ANIMAL WELFARE

The mAbs have long been produced in ascite, the hybridomas being injected into the peritoneum of rodents, proliferating and being stored in ascitic fluid that is subsequently collected. Considered unethical, this method is associated with pain in the animal and since 2010, the European legislation highly recommends *in vitro* mAb production. In accordance with animal welfare recommendations, some companies have already developed a deep expertise with *in vitro* mAb production making it as performant and even more efficient than the ascite protocol, while other companies still continue to use the *in vivo* technique.

MONOCLONAL VS POLYCLONAL ANTIBODIES

Monoclonal antibody, an infinite source

BENEFITS
- Homogeneity
- High specificity
- Quantification

For very precise targets such in therapeutics, **monoclonal antibodies** (mAbs) are essential. They are produced in cells derived from a unique clone which ensures them to be highly specific to a well-defined epitope of the antigen (Figure 1).

Accordingly, they are favoured when a high batch-to-batch reproducibility is requested, especially when a complex antigen is used as immunogen. The high specificity of the mAbs also leads to low cross-reactivity and low background response, providing reliable results in assays involving quantification (*e.g.* : flow cytometry). The possibility to choose the IgG subclass of the mAb can also be helpful (*e.g. :* secondary antibody subtype should be compatible with its primary antibody). Monoclonal antibodies take time to be generated (3-6 months) and are more expensive than polyclonal production but once a hybridoma is available the mAb production is reproducible and could be unlimited, offering high ratio of specific antibody within low timeframes and at low cost over the lifetime of the application (Figure 2).

Polyclonal antibody, for general applications

BENEFITS
- Inexpensive
- High sensitivity
- Quickly produced

Polyclonal antibodies are mixtures of antibodies coming from multiple producing cells present in the host serum that are specific for a single antigen through the recognition of various epitopes of the antigen (Figure 3).

Due to the recognition of multiple sites per antigen, pAbs are characterised by a high sensitivity and can amplify the signal from a target with low expression. They also offer a tolerance to variations of the antigen in the sample (denaturation, polymorphism, PTM or conformational changes) and of the experimental conditions (pH, salinity changes). However, due to the diversity of the antibodies produced in the polyclonal response and the use of living hosts rather than

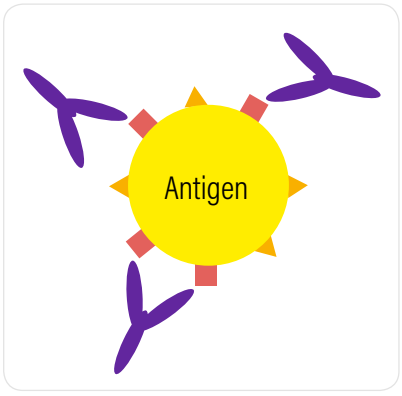


Fig.1. Monoclonal antibodies, bind to one specific epitope of the antigen

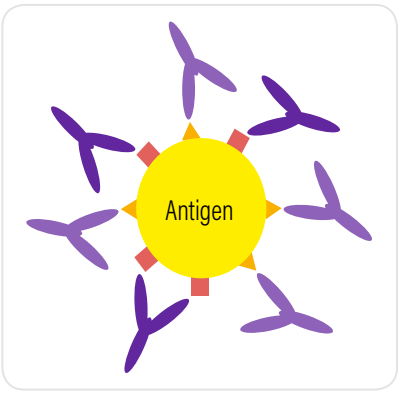


Fig 3. Polyclonal antibodies, bind to several different epitopes of the antigen

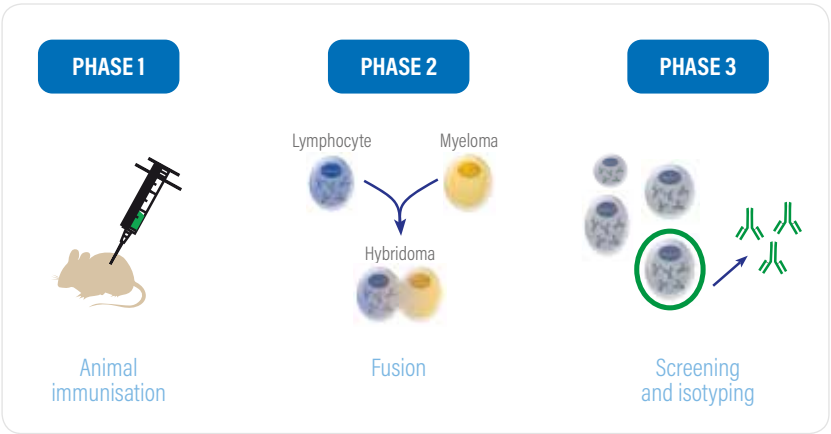


Fig 2. Production steps of monoclonal antibodies

GOOD TO KNOW

Peptides are too small to activate efficiently the immune response and must be linked to a carrier prior to immunisation.

stable cell lines, antisera characteristics may be hard to reproduce and their quantity is limited by the size of the animal and its lifetime. Compared to monoclonals, polyclonal antibodies are made up of different IgG isotypes. Easy to label and ideal to quickly capture the target protein in assays (such as Sandwich ELISA, IP or ChIP), pAbs offer the advantages of being available from multiple hosts, of being cheaper and more rapidly produced than mAbs (Figure 4).

ANTIGEN TYPES

To generate a custom antibody, the antigen (protein, peptide or complex sample) can be provided by the customer or alternatively, experts can design and synthetise it.

A good antigen must be highly antigenic in order to illicit a strong immune response : exceeds a minimum critical size (greater than 10 kDa) and shares low homology with the host components. Given these requirements it is highly recommended that a sequence alignment between the antigen and the animal host proteome be evaluated.

Protein antigens contain multiple potential Ab recognition sites that can lead to very rich and complex pAb mixtures. However, if the number of antigenic epitopes on a protein is large, this can result in poor batch-to-batch reproducibility. Additionally, some proteins are complex and expensive to produce in sufficient quantity or purity to generate useful antibodies.

Peptide antigens present a limited number of potential epitopes. They are affordable and offer the advantage of being easily synthetised at relatively low cost. They can be used to generate Abs against a protein which is not available and against PTM. However it is important to note that only sequential epitopes can be mimicked using a peptide strategy. If conformational epitopes are required, a natively folded purified protein is required.

On average, peptides of 10-15 residues are optimal for anti-peptide antibody production. As the peptide length increases so does the number of possible epitopes but also the difficulty in synthesis and purification. Peptide antigens should be chosen depending on their probability to be exposed at the surface of the protein.

Complex samples are mixtures of proteins, cellular components, whole cells, tissues or lysates. They highly stimulate the host immune response and will produce antibodies to a very large number of antigens.

SELECTING THE BEST HOST

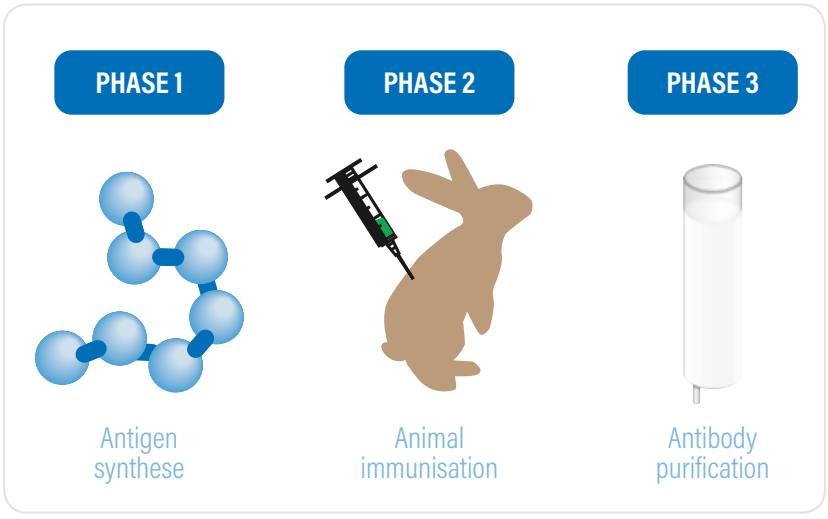
For custom polyclonal antibodies

Companies offer immunisation in a wide range of hosts. Each host presents pros and cons, regarding

IS THE ANTIGEN DIFFICULTTO PRODUCE ?

Genetic immunisation could bring a solution.

Fig 4. Antigen synthesis



ABOUT ANIMAL WELFARE

AAALAC (Association for Assessment and Accreditation of Laboratory Animal Care) is an organisation created in 1965 which promotes humane treatment of animals in scientific research around the world. AAALAC accreditation is considered the gold standard in animal welfare. Institutions (companies, universities, hospitals, governments, agencies, etc.) who have earned AAALAC accreditation have demonstrated their commitment to responsible animal care and use. Today, more than 950 organisations worldwide are accredited.

FELASA (Federation of European Laboratory Animal Science Associations) represents other common interests in the control of laboratory animal science (LAS) in Europe. FELASA recommends to strive for the 3Rs principle in Laboratory Animal Science : Replacement, Reduction and Refinement.

Other different animal welfare standards and guidelines exist and vary according to country legislation. Some certifications and controls are mandatory while others are only voluntary, the institutions choosing to be accredited or not.

the cost, the antibody quantity produced, the potential homology with the target, and the project duration. The most common hosts and their main characteristics are described below.

The **rabbit** is suitable for most applications, as this host animal offers multiple advantages: easiness of maintenance, efficient immune system and bleed volumes adapted to classical requirements. Immunising one rabbit yields 50 to 70 mL of blood, which corresponds to 10-20 mg of purified pAb on average.

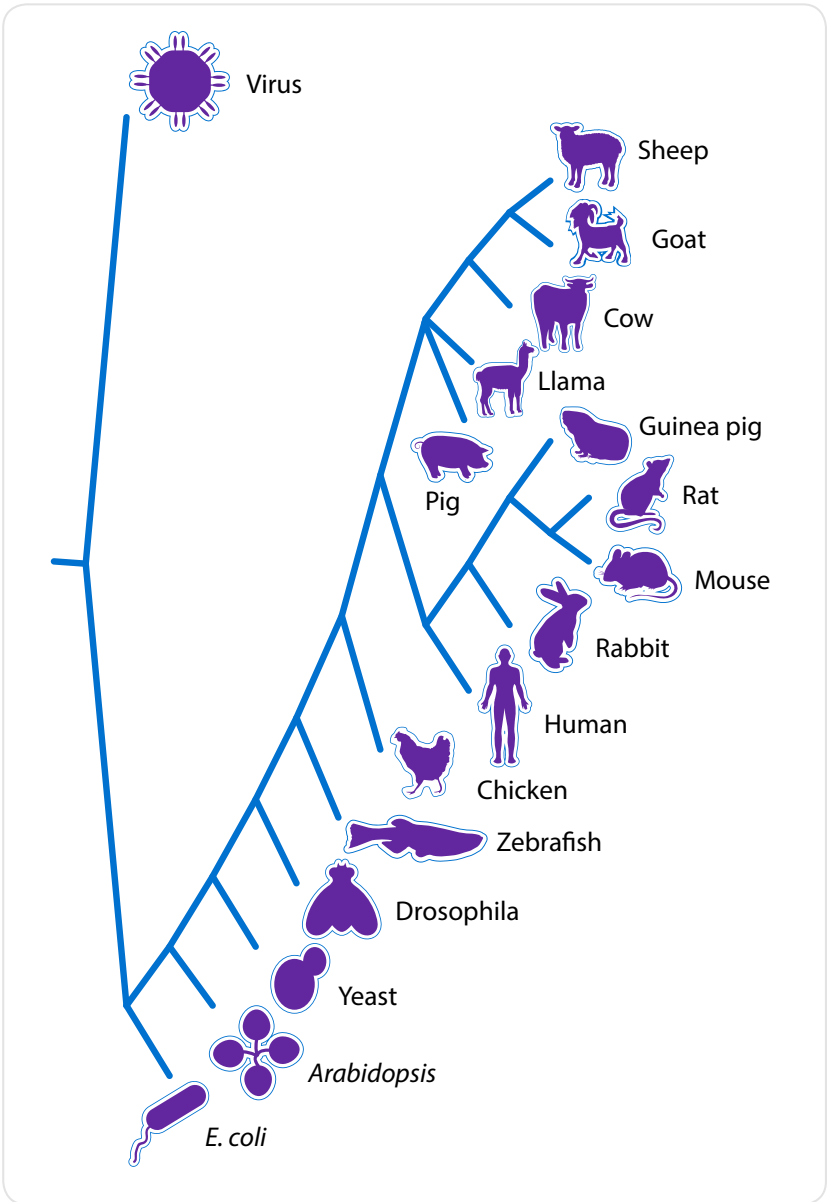
If larger antibody quantities are needed, larger animals such as **goat** or **sheep** will provide 1 L of blood volume per animal, and going to bigger hosts such as cows would increase even more the quantities of antibodies produced.

If the application requires only small quantities of antibody, polyclonal antibodies can be raised in **mice** (300 µL), **rats** (5-7 mL) or **guinea pigs** (10-15 mL), in order to benefit from lower prices without compromising the quality of the antiserum. Mice are also ideal for **mAb production** (see below).

Besides blood volume, **protein homology** is another key factor to be taken into account when choosing the host: the lower the homology between the antigen and the host proteins the better the immunisation response (Figure 5). Therefore, it is useful to perform a BLAST of the antigen sequence to evaluate whether the selected host has any homology with the antigen sequence. If the antigen is much conserved in mammals, switching to a non-mammalian animal species is a solution.

Chicken should be the preferred choice in that context. Another advantage of raising antibodies in chicken is that eggs are collected instead of blood which is preferred for animal welfare. Egg yolks contain very

Fig 5. Antigen homology



high titres of the so-called IgY antibodies, which are comparable to mammalian IgG antibodies in terms of structure, making chicken another host of choice for applications requiring large quantities of polyclonals.

Llama antibody fragments are good candidates for **therapeutic** applications that necessitate small enough molecules to be stable in vivo. Indeed, camelids produce single-chain antibodies in addition to conventional ones. The antigen binding domains of these antibodies, called VHH, are the smallest naturally occurring antibody fragments that recognise the antigens (Figure 6). Due to their small size, VHH can bind epitopes that are hidden and are relatively easy to produce in lower eukaryotes.

Finally, if a **secondary antibody** is needed in the final application, its availability must be considered: anti-guinea pig is not common, while anti-goat and anti-rat seem to suffer from a lack of batch to batch reproducibility. The most usual catalogue secondary antibodies are anti-mouse and anti-rabbit produced in goats, showing little cross-reactivity.

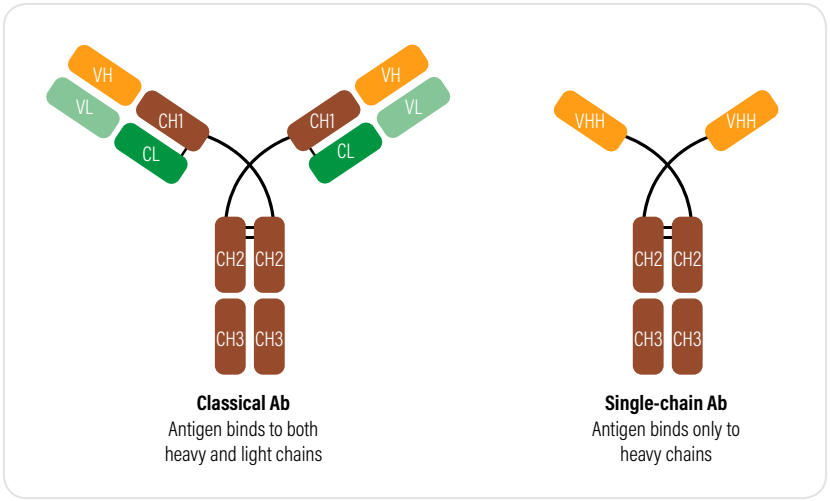


Fig 6. Structure of the lama antibodies

For custom monoclonal antibodies

Hybridomas can produce mg to g quantities of mAb by *in vitro* production. Hybridomas are created by cellular fusion of lymphocytes with myeloma and often come from the immunisation of **mice.** Murine hybridomas are developed in 16-19 weeks and cell banking allows to produce the same mAbs over time.

Rabbit mAb is a special case to mention because the fusion to generate rabbit hybridomas is patent-protected. Companies can still assist you by immunising the animal, checking the immune response and isolating the B-cells from the spleen.

Other hosts such as **rat** and **guinea-pig** may help obtaining good-quality mAbs when mice do fail.

Binding to protein A or G

IgG is the most common antibody isotype produced in mammals, while IgY is found in majority in birds. Protein A and protein G can bind with more or less affinity and specificity to the Fc region of IgG antibodies from mammals. Often used to purify, fix or detect antibodies, protein A and protein G show different affinity profiles depending on the origin of the antibody (see Table 2). ■

Origin of Immunoglobulins	Protein A	Protein G
Mouse	+++	++++
Rat	-	+++
Guinea pig	++++	++
Rabbit	++++	+++
Goat	+/-	++
Sheep	-	++
Pig	+++	+++
Chicken (IgY)	-	-

Table 2. Binding of IgG from different species to protein A and G

CONCLUSION

The choice of an antibody is a delicate task and several parameters have to be taken into account. From the "ready-to-use" catalogue antibody to the fully tailored custom antibody, many options are offered, allowing multiple applications and matching specificities. In any case, sufficient information must be provided on the material, antibodies as well as antigens, and researchers must always keep their eyes wide open on what they are buying.

DISCLAIMER

This white paper is for informational purposes only. The information contained herein represents the personal opinion of the authors only.

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