Custom Polyclonal Antibody Production

Product Description

A fully project managed service providing a customisable and adaptable approach to polyclonal antibody production. APS provides a wide range of options which can be tailored to your exact specifications. Production services can be further combined with additional contract services depending on your project requirements and available in-house resources.

Key Features:

- **Fully customisable:** Full consultation is the key to a successful project. Each project is developed to exact requirements - no off-the shelf, one-size fits all packages
- **Wide range of species:** the most common species for pAb production include rabbit, sheep, goat, mice and rats. Also available are alpaca, llama and emu (eggs).
- **UK Licensed:** All antibody production services are carried out in secure UK facilities under veterinary supervision meeting current ethical requirements, ensuring the highest quality of care for host animals, and the production of premium quality antibodies.
- **Complete confidentiality:** We understand the absolute requirement for complete confidentiality in all the custom projects. No project information will be disclosed except to clients – can be qualified with CDA/NDA's when required
- **First class customer service:** All clients can expect excellent customer care. We recognise the importance of regular communication with our clients throughout each project
- **Technical Expertise:** Over 30 years' experience in antibody production

Standard Custom pAb Production Workflow:

A typical project includes immunisation, the selected animals and their associated husbandry costs, plus bleed material (pre-immunisation, sample and production). Additional services such as ELISA testing and purification can be included as required. Please contact us at sales@lifesciencegroup.co.uk to discuss your project requirements in detail.

Prior to any project commencing, a Justification Application must be completed and returned in order to acquire the necessary veterinary approval to proceed. All schedules are also sent for pre-approval and sign-off prior to any work commencing on the project. No project will be terminated without written consent.

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Project Requirements

All custom polyclonal antibody projects are produced in the UK under Home Office licence. The Custom Polyclonal Antibody Production Unit holds a licence under the Animals (Scientific Procedures) Act. Our facilities are regulated and controlled under strict UK Home Office guidelines, ensuring a high standard of care by experienced and dedicated staff.

- **Staff**: The Custom Polyclonal Antibody Production Unit has qualified licensees to carry out procedures and to cover husbandry and animal care.
- **Plan of work**: Before any work is started, a Scientific Justification must be completed and COSHH forms for the antigen supplied. APS supplies Justification forms for completion prior to commencement of any project. Once approved, the project is logged onto a database and animals are allocated to the project. A protocol is generated and sent for customer approval. The project is then carried out according to the schedule. Any changes to the protocol need to be confirmed in writing. Should and changes be adopted during the course of a project, a new protocol is generated and sent to the customer to sign. Termination of the project is not performed until written confirmation is received from the customer.

Scientific Justification

A scientific justification is required before production can be carried out. The justification is then assessed as part of the Ethical Review Process. All requests are forwarded to the Home Office for approval. The following is a summary of requirements:

- A brief summary about the target antigen.
- An explanation as to why this specific antibody is required.
- Background of the scientific study.
- Potential benefits: It is important to explain how your work will benefit mankind. Antibodies can be made for the following purposes:
    - The prevention (whether by the testing of any product or otherwise) or the diagnosis or treatment of disease, ill health or abnormality, or their effects, in man, animals or plants.
    - The assessment, detection, regulation or modification of physiological conditions in man, animals or plants.
    - The protection of the natural environment in the interest of the health or welfare of man or animals.
    - The advancement of knowledge in biological or behavioural science.
    - Education or training other than in primary or secondary schools.
    - Forensic enquiries.
    - The breeding of animals for experimental or other scientific use.

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Antigen Selection

An immunogen is an antigen or any substance that may be specifically bound by components of the immune system. The immunogenicity of the chosen antigen is of paramount importance to the success of any custom polyclonal antibody project. The characteristics of the antigen must be determined as this will impact the choice of host and protocol used to create the antibody and the eventual success of the project.

Whatever antigen is used, there will probably be multiple epitopes against which antibodies could be generated. When choosing epitopes for your antibody production, it is best to look for those with exposed hydrophilic regions as these will be easily accessible for antibody binding. Hydrophobic regions are usually hidden within the protein.

Antigens can be derived from a variety of sources:

Peptides	Peptides are biologically occurring short chains of amino acid monomers linked by peptide (amide) bonds. APS offer custom and modified peptides together with analogues, for example phosphorylated peptides. Our **Custom Peptide Synthesis Service** includes design, synthesis and modification. Peptides are synthesised using solid-phase chemistry and fully automated multiple peptide synthesisers. APS offers peptides at a variety of purities, from >70% to 98%, dependent upon the proposed application. Peptides may also be synthesised to different lengths (typically 10-20 residues). Modifications to peptides include the addition of a functional group, such as phosphorylation, coupling to a carrier protein and labelling with biotin. Peptides are supplied lyophilised.
Proteins	Protein antigens can be either naturally occurring or recombinant. When choosing your protein antigen you should consider the following:- The size of the protein, the degree of concentration, the amount of aggregation and the relative nativity of the protein can all affect the quality and quantity of antibody produced. Generally, the larger the immunogenic protein the better. Antibodies to native proteins react best with native proteins and antibodies to denatured proteins react best with denatured proteins. For example if antibodies are to be used on Western/immune blots, or other assays where the proteins are subjected to denaturing conditions, then antibodies should be made against denatured proteins. Alternatively, if antibodies are to be used to react with a native protein or to block a protein's active site, then antibodies should be made against the native protein.
Bacteria / Viruses	Live bacteria or viruses, should not be used except as a last resort. There are many fixatives and inactivation techniques and one can generally be found that does not damage the antigenic determinants.
Plants / Yeast	Sometimes high backgrounds can be found in host animal sera for plant or yeast

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	antigens. We can supply pre-immune serum for screening for selecting candidate animals for immunisation.
Other Antigens	Small polypeptides, polysaccharides and other non-protein antigens generally need to be conjugated or cross-linked to larger, immunogenic carrier protein such as Bovine Serum Albumin (BSA), OVA and KLH to increase immunogenicity.

Antigen Preparation

With the selection of a suitable antigen being critical to the successful initiation of an immune response in the host animal, we can provide immunogen preparation services, including peptide synthesis and carrier protein conjugation.

- Antigens should always be prepared using techniques that ensure that they are free of microbial contamination. Most protein antigen preparations can be sterilized using a 0.22 micron filter.
- Where possible, antigens should be free of preparative by-products and chemicals such as polyacrylamide gel, urea, endotoxin, particulate matter and extremes of pH.
- The buffer used to re-suspend your protein is important as some buffers may have an adverse effects on the animal's welfare. The least toxic option available should be used.
- The final immunogen used is typically an oil/water emulsion so therefore detergents should be avoided if possible as these may affect the stability. SDS can be used provided the concentration is less than 0.1%.
- APS can accept antigens as either lyophilised or liquid material. Antigens may be also be provided prepared as a conjugate to a carrier protein. This is also a service option available as part of a custom project.
- **Carrier Protein Conjugation:** A standard peptide of 15-20 amino acids in length is too small to elicit an immune response on its own. As a result, it is necessary to conjugate the peptide to a larger carrier protein. We can accept antigens as lyophilised or liquid material, which can be conjugated to a carrier protein or we can provide this service for you.
- **Adjuvant:** the adjuvant, Complete Freunds, is typically used for Primary immunisation, with subsequent immunisations using Incomplete Freunds. In order to eliminate or reduce discomfort, APS pre-screens batches of adjuvant and reserves suitable batches. A number of other adjuvants are available on request.

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Species Selection

Since the selection of a host species is determined by a number of factors such as the level of immune response and volume of serum required, a wide variety of host species are available.

Rabbit	New Zealand White (NZW) and Sandy Half Lop rabbits are selected for their strong immune reactions against most antigens in a relatively short period of time. The raw antisera can be used directly in assays.
Goat / Sheep	Both sheep and goats give strong immune responses against most antigens and produce large volumes of serum. Sheep and goats are the host of choice if large volumes of antisera is required. Sheep and goats can be kept for long term projects and bleeds can be taken monthly for a period of 5 consecutive months. Our goats are from a disease-free herd (one of the only ones in Europe). This is of importance as vaccination is not effective against the following diseases: Johne's Disease (*Mycobacterium paratuberculosis*), Tuberculosis and Caseous Lymphadenitis (*Corynebacterium pseudotuberculosis*). In addition, animals are vaccinated against Bluetongue disease, Brucellosis (Brucella organisms) and *Clostridium tetani* and *Clostridium perfringens*.
Mouse / Rat	Mice and rats are used as hosts when screening potential antigens prior to immunisation into a larger host. Due to their size, they only provide small amounts of serum.
Alpaca / Llama	Camelids such as alpacas are unique in producing antibodies composed solely of two identical heavy chains which makes them of great interest for basic research and therapeutic applications. Alpaca antibodies are robust, with the ability to withstand elevated temperatures and a wide pH range, extending their within *in vivo* and *in vitro* applications. Alpaca antibodies can survive the harsh environment inside cells, retaining the ability to seek out targets. This is of particular importance in the field of gene therapy and biosensor applications.
Chicken	Should be considered as the host if it is important for the phylogenetic relationship between the antigen donor and the antibody producer to be distant. Chickens transfer high quantities of immunoglobulins, (IgY), into the egg yolk, which eliminates the need for invasive bleeding procedures. The resultant antibody will need to be purified before use.
Emu eggs	Birds (most commonly chickens), offer a non-invasive production source of antibodies. Following immunisation, the antibodies can simply be collected by harvesting the eggs and purifying the antibodies from the egg yolks – to remove lipids which can interfere with assays. An emu egg is typically 10-15 times larger than a chicken egg. **Note:** Availability is seasonal, from October to March.

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Immunisation Schedule

One the most suitable host has been chosen for a Custom Polyclonal Project it is necessary to consider the design of the immunisation protocol. Not all host species elicit the same immune response to a specific antigen. APS has developed a series of host specific protocols enabling the optimum immune response over a specific period of time. These protocols may be modified to suit individual circumstances following discussion between the end user and the technical team. Full details of the immunisation schedule are supplied to the customer prior to the commencement of the project. Any changes in the protocol are carried out only with the agreement of the customer following discussions with the technical team.

A standard immunisation schedule for pAb production is 77-days for rabbit and 98-days for larger animals. Schedules can be customised. Should you require a longer schedule that the standard 77-days, additional husbandry costs apply. These are charged on a per-day basis. Veterinary consultation is available at any time before projects commence.

What does a standard 77-day schedule look like?

A standard 77 day schedule for Rabbit:

Day	Procedure
0	PRE-IMMUNISATION TEST BLEED - 10 ml
0	Primary Injection
14	Booster Injection 1
21	TEST BLEED 1 – 10 ml
28	Booster Injection 2
35	TEST BLEED 2 - 10 ml
42	Booster Injection 3
49	TEST BLEED 3 - 10 ml
56	Booster Injection 4
63	TEST BLEED 4 - 10 ml
70	Booster Injection 5
77	FINAL BLEED (Exsanguination)

Please note: Sufficient antigen must be provided in order to meet the desired immunisation schedule for the selected species.

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How much antigen do I need to supply?

We recommend the following volumes of antigen be provided for standard antibody projects:

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 – 3,000** 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 – 3,000** µg	500 – 750 ml with harvest bleeds also available to increase yield

Please note: Due to natural variations, exact volumes of serum cannot be guaranteed. Spleen preparations can also be performed.

In order to monitor the effectiveness of the protocol being followed and the immunogenicity of the antigen, test bleeds are taken at regular, predetermined intervals. Following monitoring of these test bleeds by ELISA the immunisation protocol may be modified, if required.

ELISA monitoring of Test Bleeds

ELISA monitoring of test bleeds during the progress of a Custom Polyclonal Project is a simple and convenient way to follow the development of the immunoreactivity in each host during the immunisation protocol. During this process, the test sera is coated onto adapted microtitre plates and several parameters are reviewed including:

- Antigen concentration and serum dilutions
- Incubation time and temperature for the immune reaction to occur
- Dilutions of the peroxidase-conjugated secondary antibody

ELISA screening may be carried out by APS or then the test bleed samples together with a pre-immune sample bleed from each host may be returned to the customer in order that screening may be carried out in their own facility.

Project Termination

Following the successful completion of the Custom Polyclonal Project protocol the terminal bleeds, processed into serum, are supplied to the customer in entirety. The resulting serum will contain the whole antibody as well as other protein types. Whole antisera may be suitable for use in many

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applications but it may be necessary to improve specificity by applying purification steps to the whole serum.

Antibody Specifications

The following parameters will be reports on a Certificate of Analysis for each antibody (when supplied as purified material). Concentration, label, buffer, volume and any preservative, as required.

Storage & Handling

Recommended long term storage is -20° C.

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 at least 2 weeks. For long term storage, it is recommended to aliquot the antibody and store at -20°C.

Shipping

Antibodies are supplied as frozen material and will be shipped frozen on dry ice.

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

- Sera will be 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.

Additional Contract Services

Polyclonal antibody production can be combined with additional services including:

- Peptide design and synthesis. We specialise in the production of peptides for the raising and purifying of antibodies.
- Antibody labelling including conjugation, tagging and other modifications.
- Downstream processing, purification and protein analytics.

Regulation of the use of animals in research

All antibody production services are carried out in secure UK facilities by qualified licensees, under UK Home Office licensing to ensure the highest quality of care for the host animals, and the production of premium quality antibodies. All procedures are performed under veterinary supervision, meeting current ethical standards according to the Animal (Scientific Procedures) Act 1986 (ASPA), which has been revised to transpose European Directive 2010/63/EU. Central to the ASPA is a cost-benefit assessment which must be applied before any research project involving animals can go ahead.

Ownership

Antibodies remain the property of the client.

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Support

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

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