

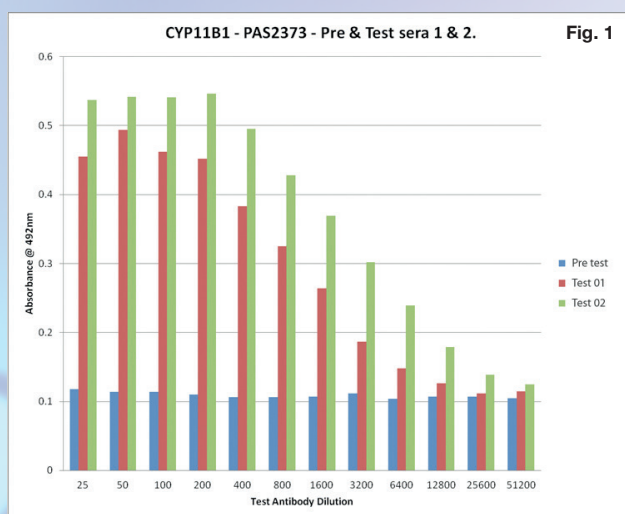
Overview Anti-Peptide Antibodies

Severn Biotech have been involved in producing polyclonal anti-sera **since 1995**, this came about as interest grew in making anti-bodies to peptide molecules. Since then we have been involved with a number of **drug development programmes** which involved the use of polyclonal anti-body projects.

The majority of antibodies produced are from NZ white Rabbits which are a reliable source of anti-sera. This makes up volumes in excess of 60ml of final product excluding test sera collected throughout the project, additionally making up initial test volumes. The NZ Rabbits are Barrier reared animals which are pathogen free. This ensures a greater reproducibility often giving higher antibody titres with low background interference.

NZ White rabbits are pre-immunised with Freund's complete Adjuvant (FCA).

The addition of a **Peptide molecule** which makes up the conjugate, normally with a carrier protein, such as **KLH** (keyhole Limpet Haemocyanin) is used in subcutaneous immunisation to elicit the immune response in NZ white rabbits. This is carried out over a period of 3 weeks and is monitored throughout by ELISA testing against the Peptide antigen. Serial dilution of the test sera indicates the level of antibodies present and the sensitivity of the ELISA test to the Antigen.



The test monitoring by **ELISA** gives an indication of the antibody concentration over time with levels reaching a plateau within 70 days. Harvesting and continuous monitoring, takes place during this period, to obtain

maximum anti-body titre levels. Once established maximum levels are harvested and anti-sera collected. See graph.....fig.1

The project follows a set pattern or protocol which can be altered to suit the requirements of the anti-sera levels and harvesting. This is normally complete within 77 days, however this can continue with some projects running on dependent upon the quantity and anti-body levels necessary.

Project Protocol

The normal standard **protocol is a 77 day** with 3 test sera collections during the immunisation period. Final and harvesting test sera levels are also conducted and ELISA tests performed at these stages. If initial immunisation is unsuccessful the project is halted immediately. So un-successful testing screens out projects which do not achieve anti-sera titres within the initial 3 week stages.

Additionally, successful titres seen from the initial tests are borne out by ELISA testing continue to harvesting with additional **booster immunisation** taking place after 65 days. Harvesting then takes place as polyclonal levels have been seen to maximise. Projects are then continued or halted at this later stage pending requisite anti-sera levels. The whole process is **licensed** and monitored by the **home office**.

Antibody Selection

Anti-bodies can be purified by using **Protein A columns** to isolate the **IgG** fractions from the harvested material, this is done to remove any blood proteins which are not required and concentrate the antibody fraction. Further, specific, antibody purification may also be facilitated by removal using Affinity Purification columns which are made using the actual peptide bound to an Agarose bead. Bespoke affinity columns made this way offer selective removal and depletion of anti-sera allowing the removal of actual specific peptide antibodies from the whole sera.

Using **Affinity purification** concentrates the antibody of interest and removes IgG molecules which are not specific or required. Although anti-sera titres will be depleted the antibodies purified are much more specific and therefore more active against the peptide sequence of interest at a much lower concentration. Thus a higher specific ratio of anti-peptide antibody to Peptide!

Peptide Design

The peptide sequence is ultimately important in the design of the **antigen, epitope or hapten** necessary to furnish a responsive antibody molecule, useful as a **reporter, diagnostic, or therapeutic**. This information can be the difference necessary! At Severn Biotech we have been helping design peptides for many years and have discovered sequences useful in distinguishing sp. where there is a high degree of homology. **It's all about the Peptide!!**

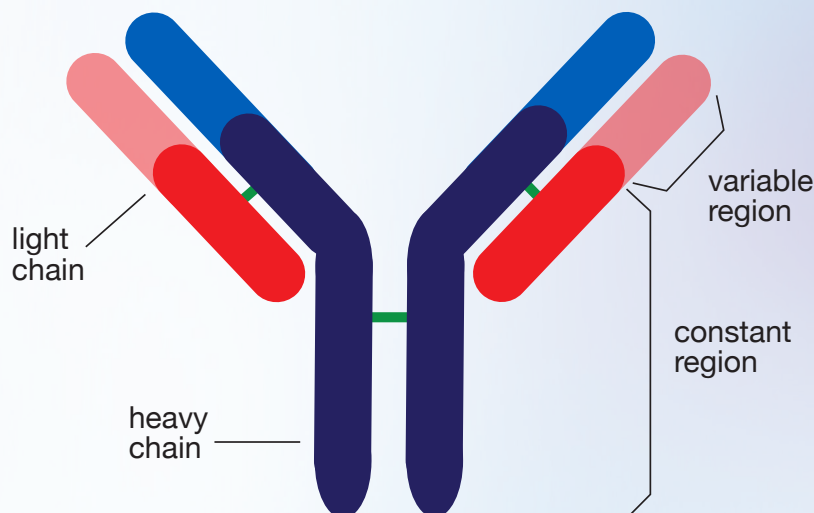
The majority of peptides will require conjugation to a carrier protein which is **highly immunogenic**, this is required to improve the response and increase antibody titres. There are a number of these which can be used and we can aid with the choice, dependent upon the desired result.

What Next

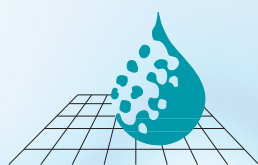
If you have a project you would like us to discuss then contact Severn Biotech Ltd sales office and we will provide you with a complete **information package** which will help allow you to plan your antibody project and achieve the desired results. Projects often require two NZ white rabbits and require Severn Biotech to work closely with the client to ensure we deliver what will give the best results. We have years of experience and understand that each antibody project is unique.

Severn Biotech: Anti-Peptide Antibodies. (ISO9001:2008)

- Specific Polyclonal Antibodies made to your peptide sequence
- Peptide Antigen conjugated to Carrier Protein (KLH,BSA et al) mediated response in NZ White Rabbits
- Peptide Epitope design service , leading to Synthesis and Conjugation of synthetic Epitope
- Protein A Purification of IgG. titres
- Bespoke Thio-Link TM Affinity Columns made with Specific Peptide of interest.
- Affinity Purification of Peptide Anti-sera produced.
- Elisa Tested sera with serial dilution response.
- Collated data information/Affinity purification column/Anti-sera provided
- Also available Custom Monoclonal Antibodies
- Home office Licensed. Gold standard.



For further information please contact Severn Biotech Ltd



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