Custom Polyclonal Antibodies against Synthetic Peptides

The use of synthetic peptides as immunogens is generally applied where either the complete protein is not available in sufficient quantities to carry out an adequate immunization protocol, or to obtain antibodies able to recognize only specific regions of a polypeptide chain. Synthetic peptides offer the opportunity of a very fast shortcut to overcome the lack of protein. The selection of the amino acid sequence of the peptide is a crucial step toward the success of such a strategy. Our technical staff will assist our customers with the design of the best peptide sequences. We suggest a length of 15 amino acid residues, and this service is free of charge.

In some cases the use of more than one peptide from a given protein chain is recommended. After synthesis, the peptides are combined and used as a single antigen toward the preparation of antibodies able to recognize all the peptides. Through such an approach, the resulting antibodies have a higher capability to recognize the intact and native protein chain.

Why the customer should choose polyclonals Abs against synthetic peptides?

- For antibodies against specific regions of a protein
- To discriminate between two or more isoforms of a protein
- Antibodies for Western Blot experiments (denatured condition)
- Antibodies for ELISA experiments (indirect, sandwich and competition test)

Peptide conjugation

To increase the immunogenicity, it is necessary to link the peptide sequence to a larger protein molecule, which serves as a carrier and it should be higher than 20,000 daltons.

Most of the immunopeptides produced by Primm are conjugated with carriers such as OVALBUMIN or KLH. Normally we conjugate about 3/5 mg of peptide.

Screening and quality control

Titer and quality of the polyclonal antibodies are verified by ELISA using the free peptide. Results of the test are supplied with the antibodies.

Our protocol includes:

- Synthesis and Purification of 1 or more peptides
- Peptide quality control: HPLC & Mass Spec Analysis
- Carrier Conjugation
- Immunization of rabbits, mice or rats

What we deliver

- Total serum of the animal, 50 ml out of each rabbit
- Pre-immune sera, 2 ml out of each rabbit
- Left-over free peptide (2-5 mg)

Product specifications

Able to specifically recognize the antigen (free peptide or peptide conjugated to a different carrier protein) at a dilution no lower than 1:10,000 according to ELISA test.

Dual Species Custom Polyclonal Antibody Service

Immunization of two species (2 rabbits & 4 mice) obtaining two independent polyclonal preparations.

Double species means

- Dual species benefits
- Higher chances of generating antibody preparations for multiple applications
- Mouse serum is generally very clean, so mice polyclonal antibody is an excellent choice for immuno histochemistry studies
- Rabbit serum is often very complex, for certain applications rabbit polyclonals need to be affinity-purified. Mouse polyclonals do not need (and cannot) to be purified

Polyclonals from 2 species can be considered as two separate antibody preps. This provides twice the reagents for the customer with increase probability to generate useful antibodies.

This service provides the customer the possibility to go from mouse polyclonals to monoclonal antibodies generation.

Primm specializes in production of the ideal antigen from peptides to recombinant proteins and also accepts custom antigens.

Primm provides two different procedures:

1. PAbs against synthetic peptide

- peptide design & synthesis
- carrier conjugation (KLH or Ovalbumin)
- deliver of free remaining peptide

2. PAbs against recombinant protein

- choice of different cloning plans
- gene cloning, protein expression and purification
- deliver of free remaining recombinant protein and plasmidic DNA

QUALITY GUARANTEED

For both protocols
2 rabbits & 4 mice immunization (about 45-50 days protocol), ELISA test and minimum titer is guaranteed.

Monoclonal Antibodies Custom Service

Our efficient monoclonal antibody production service includes immunization of 3 mice with antigens either provided by the customer or produced by Primm (recombinant proteins - synthetic peptides). Approximately 2 mg of protein are necessary for immunization and analysis.
This service allows the generation of monoclonal antibodies through somatic fusion and the possibility to choose either 2 or more hybridomas after the first screening. Primm can provide both the cell lines and the purified monoclonal Abs. A cell banking service is also available for the storage of the desired clones.

The development and production of monoclonal antibodies goes through different phases:

Production of antigen

recombinant protein

- Gene cloning
- Protein expression
- Protein purification

synthetic peptide

- Synthesis of about 10 mg of peptide, purity >70%
- Conjugation of 3-4 mg of peptide to a carrier protein (as antigen)
- Conjugation of 1-2 mg of peptide to a different carrier protein for ELISA screening

Phase I (1.5 months)

- Immunization of 3 BALB/c mice with the antigen
- ELISA testing of the immune response and selection of the animals for subsequent spleen cell fusion
- Delivered products: ELISA tests

Phase II (2 months)

- Splenectomy and fusion of splenocytes with myeloma cells to isolate hybridoma cells
- Selection of hybridoma by ELISA: freezing of the more positive mother clones
- Delivered products: supernatants of mother clones

Phase III (1-1.5 months)

- Cloning and subcloning of positive clones (2 or more) by limiting dilution (subcloning and expansion of single clones each one producing single monoclonal antibody)
- Freezing of the hybridomas
- Viability, productivity and stability assays after thawing of one aliquot
- Delivered products: frozen hybridomas (sub-clones + mother clones)

What we deliver

- 2 or more hybridomas cell lines – single clones (selected by the customer), each one able to produce a single MAb
- The cell lines are permanent sources. Upon cell cultivation they produce MAb in the culture supernatant

Product specifications

- Stable cell lines
- Aliquots can be stored at our facility

Phase IV (1-1.5 months)

- Production - cell culture *(in vitro)* or ascitic fluid - and purification of MAb (the purification is performed by Immunoaffinity chromatography specific for either IgG or IgM)

What we deliver

- Purified monoclonal antibodies obtained through either liquid ascitic production or cell cultivation of the hybridoma cell lines at our facility

primm

Solutions for Your Research

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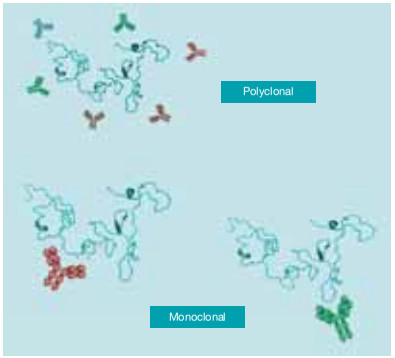
Custom Antibody Services



The service we offer is very complete starting from rabbits, mice and rats. The different formats provided by Primm depend on the antigen of choice:

- Custom antibodies against proteins or antigens supplied by the customer
- Custom antibodies against recombinant proteins
- Custom antibodies against synthetic peptides
- Custom antibodies against phospho-synthetic peptides

Related to the antibody production Primm also offers a wide range of services including:

- Free antigenicity analysis
- Free and accurate assistance for the selection of the best antigen
- Development and production of the antigen for immunization if not provided by the customer
- Antibody purification by affinity chromatography
- Antibody labeling
- Immunometric assay
- Dual species custom polyclonal antibodies

The quality leader for custom biotech services for the scientific community

- **Peptide synthesis**
- **Protein analysis**
- **Gene cloning and expression**
- **Gene synthesis**
- **Custom Antibodies**
- **Animal studies**

For a competitive quotation:
quotes@primmbiotech.com

Custom Polyclonal Antibodies against Recombinant Protein

Primm has developed a very competitive service based on an optimized fast and efficient technology to produce recombinant protein for immunization. Directly from the nucleotide gene sequence or gene accession number (human/mouse/rat origin), Primm will proceed to the isolation of the cDNA clone.

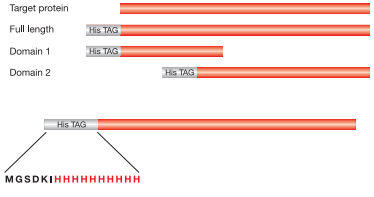
The complete service includes:

- Gene cloning
- Complete DNA sequencing of the clone
- Expression in *E. coli* as a fusion protein
- Purification of the recombinant protein
- Generation of polyclonals

In the event that a reliable RNA source is not available (for unusual organisms or species), the customer is requested to supply the gene.

Depending on the protein size and features, Primm will clone and express the full length gene. Alternatively, a selected domain of 50-300 amino acids will be chosen for expression and immunization, we will then proceed with the form that is best produced.

To select the best domains Primm is always open to take into consideration specific needs and preferences suggested by the customer.



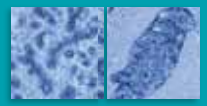
Recombinant proteins or protein domains will be produced as His-Tag fusions from *E. coli* cells and purified in quantities sufficient for the immunization protocol and for ELISA or Western Blot testing; the proteins are produced as *"standard grade"* in denaturing buffer.

What we deliver

- Total serum of the animal, 50 ml out of each rabbit
- Pre-immune sera, 2 ml out of each rabbit
- Remaining recombinant protein (0.5 mg max)
- Plasmidic DNA (3 µg)

Product specifications

Able to specifically recognize the recombinant protein at a dilution no lower than 1:10,000 according to ELISA test



For project pricing:
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ph. 800 893 4388

Polyclonal Antibodies against Synthetic Phospho-Peptide

Phosphorylation is the addition of a phosphate (PO_4) group to a protein molecule or a small molecule. Its prominent role in biochemistry is the subject of extensive research. Phosphorylation usually occurs on serine, threonine, and tyrosine residues in eukaryotic proteins. Antibodies can be used as powerful tools to detect whether a protein is phosphorylated at a particular site. Antibodies bind to and detect phosphorylation-induced conformational changes in the protein. Such antibodies are called phospho-specific antibodies. They are becoming critical reagents both for basic research and for clinical diagnosis.

Phospho-specific antibodies are generated using, as an antigen, a peptide containing a phosphorylated amino acid residue, Primm helps you to select the best peptide sequence. We suggest:

- **A short peptide to increase the relevance of phosphorylated aminoacid**
- **Phosphorylated amino acid residue either at N- or C-terminus**
- **Cys residue added for selected carrier conjugation via disulfide bridges**

Phosphorylated protein

GQILKELIQNAEDAGAVGRFGK**KNRSVYpHK**TDVPCFSGDQIGMLDPHQTLF
GPHESGQCWNLKDDSKEISELSDQFAPFVGIFGSTKETFIN

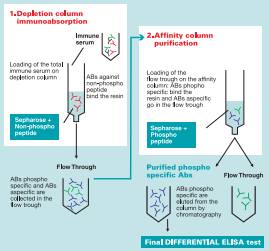
↓ Peptide selection

Cys-KNRSV**Yp**HK

Differential purification of anti-phospho antibodies

Primm separates the immunoglobulins recognizing phopho-peptides from the immunoglobulins recognizing the non phospho-peptides.

Phospho Specific Antibody



Primm Phospho Specific Polyclonal Antibody production includes:

- Immunization of two rabbits with a phospho-synthetic peptide conjugated either to OVA or KLH
- Synthesis of the corresponding non-phospho peptide for preparation of depletion column and for differential ELISA test
- Standard immunization schedule (5 injections - about 1.5/2 months)
- Final bleed (40-50 ml/rabbit)
- Preparation of depletion column + immuno absorption
- Preparation of affinity column + immunoaffinity purification
- Phospho polyclonals positivity is guaranteed at a dilution of the purified antibodies equivalent to 1:10,000 as compared to pre-immune serum in differential ELISA test