



2. Custom polyclonal ANTI - PEPTIDE antibody

Immunogen

- peptide design
- peptide synthesis
- conjugation

Immunization

- animals
- protocols
- volume of serum (per animal)

Related Services

- animal selection
- quality control : Elisa test and Western Blot
- purification

Standard and Package Services

Using synthetic peptides as immunogen is the simplest way to produce antibodies against non-purified proteins or against putative protein sequences derived from DNA sequence information. This method is also useful when isolation of the antigen is difficult and time consuming or if the antigen is a member of a large protein family. The understanding of your needs following our discussion, will allow us to provide the best peptides for your project.

Immunogen

■ Peptide design

To perform the peptide design, the following information will be required:

- Protein name
- Sequence (FASTA format)
- Domains of interest (N-ter or C-ter, cytoplasmic or extracellular)
- Specificity required by comparison to other proteins
- List of homologous proteins
- Application (WB, IHC, IP...)

Based on the supplied information on the protein an analysis of the different parameters such as hydrophobicity, antigenicity, solvent accessibility, post-translational modifications, presence of transmembrane domains and a blast of sequences will be performed to design the most relevant peptides.

A list of designed peptides will be sent to you.

For your information

Using 2 or 3 peptides will increase the probability to obtain high reactive and specific antibodies which may work for different applications (WB, IHC, ...).

■ Peptide synthesis

All peptides are produced with the Fmoc strategy on solid phase and analysed by HPLC and mass spectrometer. The quantity to be synthesized depends on the service chosen (Standard or Package service).

Extra fees will be charged for longer peptides and higher quantity and purity.

We advise you according to our experiences

To co-inject 2 to 3 peptides for the following reasons:

- The immune system is even more stimulated
- More than one epitope of the protein can be recognised

You will receive, for each peptide:

- All remaining peptides at the end of the project.
- A technical data sheet of your peptide.
- Mass spectrometer and HPLC profiles.

■ Conjugation

Usually, we conjugate the peptide to a carrier protein via a cysteine residue. If the peptide sequence does not contain a natural cysteine, we add a cysteine at N-ter or C-ter sequence of the peptide, otherwise specific conjugations are used.

Carrier proteins used: KLH – **Keyhole Limpet Hemocyanin**, BSA – **Bovine Serum Albumin**, OVA – **Ovalbumin**.

Quantity of peptide conjugated: 5 mg (10mg for goat projects).

Immunization

■ Animals

Covalab can offer a wide range of predefined protocols which can be adapted to your needs :

Species	Strain	Duration of the standard protocol (days)	Injections
Rabbit	New - Zealand	53/67/88	ID & SC
Chicken	Isa - Brown	90	SC & IM
Guinea Pig	Dunkin Hartley	88	SC
Rat	Sprague Dawley	81	SC
Mouse	BALB/C	67	SC & IPr
Goat	Alpine Chamoisée	88	SC

■ Protocols

At the end of the standard protocol, you can request :

- The final bleed
- One or several intermediate bleeds to obtain higher volume of serum
- An extension of the standard protocol to improve the serum's immune-reactivity

Steps	Protocols							
	Rabbit	Rabbit	Rabbit	Chicken	Guinea Pig	Rat	Mouse	Goat
	53 D	67 D	88 D	90 D	88 D	81 D	67 D	88 D
Transfer of animals to the experimental zone	-3	-3	-7 to -3	-6	-7 to -3	-7	-3	-7 to -3
Pre-immune Bleed	0	0	0	0	0	0	0	0
First injection (*)	0	0	0	0	0	0	0	0
Second injection (*) (1 st boost)	14	14	21	16	21	14	14	21
Third injection (2nd boost)	28	28	42	30	42	28	28	42
First bleed	39	39	53	55	53	39	39	53
Fourth injection (3rd boost)	--	42	63	55	63	56	42	63
Second Bleed	-	53	74	69	74	67	53	74
End of the standard protocol of immunization ⁽³⁾	53	67	88	90	88	81	67	88

(*) For the first injection the antigen is mixed with the complete Freund's adjuvant. For the other injections the in complete Freund's adjuvant is used.

(3) The final bleed is performed automatically at the end of the protocol. For any extension, please inform us one week before.

		Protocols							
		Rabbit	Rabbit	Rabbit	Chicken	Guinea Pig	Rat	Mouse	Goat
		53 D	67 D	88 D	90 D	88 D	81 D	67 D	88 D
Volumes (ml)	1 st Bleed	4 - 5	4 - 5	4 - 5	0,5 - 2	0,5 - 1	0,5 - 1	15 - 30 µl	5
	2 nd Bleed	10 - 15	4 - 5	4 - 5	0,5 - 2	0,5 - 1	0,5 - 1	15 - 30 µl	20
	3 rd Bleed	-	10 - 15	10 - 15	0,5 - 2	0,5 - 1	0,5 - 1	15 - 30 µl	200
	Final Bleed	50 - 70	50 - 70	50 - 70	10 - 20	4 - 8	4 - 8	150 - 300 µl	1000

For your information

At the beginning of each project, you will receive the immunization protocol describing the timescales for the different injections, bleeds and shipments.

Related Services

■ Animal selection

To select the best rabbits for your project, the pre- immune bleeds can be requested.

ELISA

Each sample will be tested using ELISA technology. These tests will allow you to follow the evolution of the immune response during the immunization.

Reporting

For each test, you will receive an intermediate report showing the serum's titers and the evolution of each animal's immune-reactivity. These data with your results will allow you to make the best decision.

Western Blot (WB)

To perform WB, please provide us with :

- Purified protein : 15 - 20 µg
- Cellular Lysat containing the protein of interest : 100 - 500 µg
- Positive control (if any)
- Molecular weight of the protein to be detected

■ Immunopurification of Anti-peptide antibodies

This purification will enable you to obtain specific and highly purified antibodies against your peptide(s) which is useful for IF and IHC applications. This service consists of:

- Conjugation of the peptide(s) on agarose beads
- Through flow of the serum on the column (5, 10, 25, 50 or 100 ml of sera)
- Elution of the immune-adsorbed antibodies
- Control of the immune-reactivity of the antibodies by ELISA test

For your information :

Multi-peptides projects: immunopurification against additional peptide(s) can be requested and will be charged.

The purified antibodies are provided in Tris 1M pH 8, Azide 0.02% buffer. (If you do not wish azide to be added, please inform us in advance).

Immunopurification of IgY from Egg yolks is also available. (See p.31)

At the end of your project, you will receive the purified antibodies and the corresponding report.

Summary : « Rabbit custom antibody »

Standard Service

Peptide design

Synthesis of 1, 2 or 3 peptide(s) (<16AA, >70 % purity, 5 - 10 mg)

Conjugation to a carrier protein (KLH, OVA, BSA)

Immunization of 2 animals according to the protocol of your choice

Package Service

Peptide design

Synthesis of 1, 2 or 3 peptide(s) (<16AA, >70 % purity, 20 - 25 mg)

Conjugation to a carrier protein (KLH, OVA, BSA)

Immunization of 2 animals according to the protocol of your choice

ELISA test for each bleed.

Immunopurification of 5 ml of serum.

Shipment of the immunopurification's column