From Research to Discovery

covalab
R&D in Biotechnology

Antibody Development Services

Monoclonal antibodies

Services

From project design to antibody production

Custom projects

Protocols adapted to your requirements

Technical support

Advice by experts in immunology

Ethics

Approved Health & Safety procedures

www.covalab.com

PROTOCOL OVERVIEW

I

Immunisation

4 mice or 2 rats
90-day protocols

Monitoring

ELISA assay on each test bleed
Comparison of sera immunoreactivity over time

IMMUNISATION

You choose the animal from which splenocytes will be extracted.

II

Preparation

Final boost
Splenocytes extraction

Fusion

Fusion of spleen cells with myeloma cell line
Culture in HAT selective medium

FUSION

III

Screening

ELISA tests on growing cell hybridomas
Cell expansion from each positive well

Confirmation

ELISA tests to confirm hybridoma stability
Partial isotyping *(IgM or IgG)* and freezing

SCREENING

You choose one or several positive hybridomas for cloning

IV

Cloning

Cell seeding by limiting dilution
ELISA tests on the supernatant of cell cluster-containing wells
Choice of up to 6 positive clones for cell expansion

Finalisation

ELISA tests to confirm antibody production stability
Complete isotyping *(Isotype, class, subclass and light chain isotype)* and freezing

CLONING

You choose the final clone from each selected hybridoma

IMMUNOGEN

PROTEIN

To perform all the steps of the protocol *(immunisation, development of the screening method, immunoreactivity monitoring, screening of hybridomas as well as antibody production assays if required)*, our immunisation protocol requires **few milligrams of the immunogen** *(recommended concentration: 1 mg/mL)*, which can be as:

Protein

In solution
Lyophilised
Within polyacrylamide gel

Microorganism

Inactivated virus
Inactivated bacteria
Inactivated yeast

Other

Cell or tissue extracts
Others *(please contact us)*

PEPTIDE — POST-TRANSLATIONAL MODIFICATION

Design

We use our in-house expertise to design **the most relevant peptides**.

We analyse **physicochemical properties** to predict immunogenicity and tertiary structure among others.

We ensure **minimal cross-reactivity** with non-relevant proteins by analysing their homology with other sequences.

For anti-PTM antibodies, we make sure the immune response is **optimal against the expected modification**.

Synthesis

Peptides are synthesised using solid phase Fmoc chemistry, and analysed by HPLC and mass spectrometry.

Length: up to 15 amino acids

Quantity: 20 to 25 mg

When developing an **anti-PTM** antibody, we synthesise one modified peptide as well as **one unmodified control peptide** to ensure the specificity of the resulting antibody for the modification.

Conjugation

Conjugation is usually carried out on a **cysteine residue** *(which can be added to the N- or C-terminus of the peptide if required)*, but other techniques can be used if necessary.

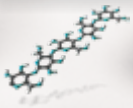
After synthesis, peptides are conjugated with one of the following carrier proteins:

KLH
Keyhole limpet hemocyanin

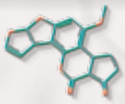
OVA
Ovalbumin

BSA
Bovine serum albumin

HAPTENS



Carbohydrates



Chemicals & Toxins

Natural or modified nucleotides

DEVELOPMENT OF THE SCREENING METHOD

An ELISA protocol is set up to measure the serum immunoreactivity during the immunisation procedure as well as the production of antibodies by hybridomas after fusion. The immunogen[1] is coated onto microtitre plates and several parameters are optimised including:

- **antigen concentration**
- **serum dilutions**
- **incubation time**
- **temperature of the immune reaction**
- **dilutions of the peroxidase-conjugated secondary antibody.**

Follow the evolution of the sera immunoreactivity and make the right decision!

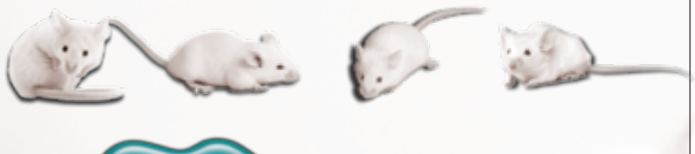
(1) For anti-peptide and anti-PTM projects, one screening method is developed for each peptide involved.

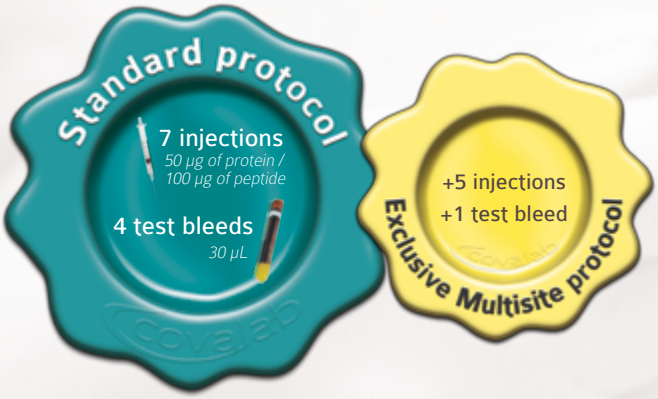
DETAILED PROTOCOL

IMMUNISATION

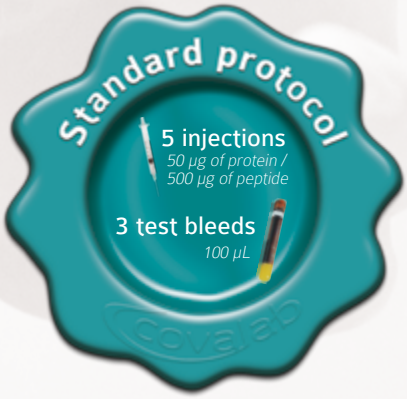
I The first step of the development procedure consists in injecting the immunogen into the host animals to generate an immune response, according to our exclusive 3-month protocol. Depending on the host species, the number of injections per animal has been optimised to generate **the best immunoreactivity / quantity of immunogen ratio**.

4 BALB/c mice





2 Wistar rats



Each test bleed is assayed according to the detection method developed for the project. Our immunisation protocols are adjustable according to the immunoreactivity of the sera. In case it is too low, additional injections and/or bleeds can be requested.

All experiments are undertaken by experienced and authorised staff following Health and Safety procedures, established according to the French legislation governing the use of animals in experiments. Our animal house is registered under the reference No C21 464 04 EA.

You receive:

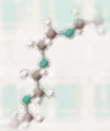
- Test bleeds so that you can run tests in your specific conditions in order to choose the most suitable animal to perform the fusion step.

Our guarantee:

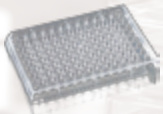
- The remaining animal(s) are kept in the animal facility to perform additional fusion(s) if necessary.

FUSION

II After having selected the most suitable animal according to both our ELISA-tests and your own results, we proceed to the extraction of its immunoglobulin-secreting lymphocytes. These cells are subsequently fused with immortalised murine myeloma cells to generate **hybridomas** which are then cultured under selective conditions.



Fusion between **splenocytes and myeloma cells** in the presence of PEG

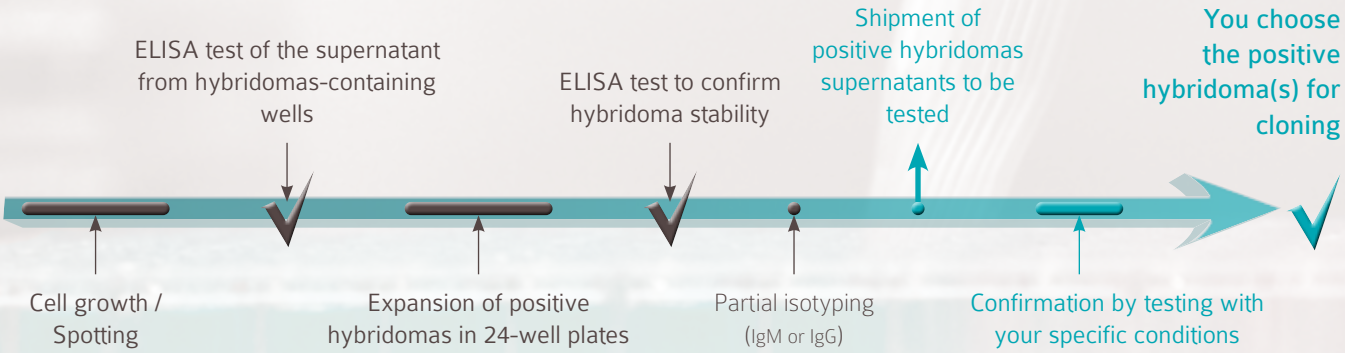


Cell seeding and culture in **Hypoxanthine-Aminopterin-Thymidine (HAT)** selection medium

SCREENING

III

After several days of cell culture, the plates are screened to identify growing cells, indicating the **presence of hybridoma cell lines**. To ensure their antibody secretion, the corresponding supernatants are assayed using the screening method developed previously. At the end of this step, positive hybridomas are still made of multiple clones **which must be isolated**.



You receive:

- Supernatant from each positive hybridoma to run tests in your specific conditions so that you can choose one or several hybridomas to be cloned.

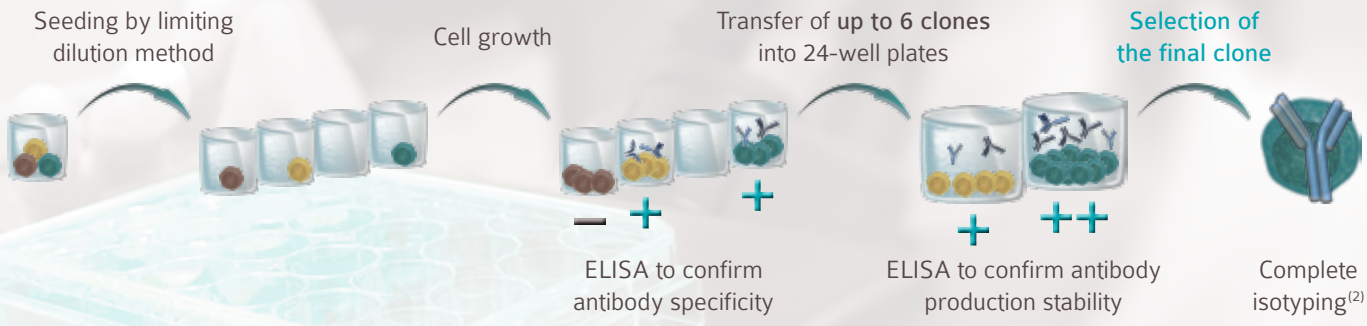
Our guarantee:

- Up to 10 positive hybridomas (at polyclonal stage)are kept frozen in liquid nitrogen to perform additional cloning steps if necessary.

CLONING

IV

The final step of the development procedure consists in seeding the selected hybridomas using the limiting dilution method, in order to **isolate the clones from each other**. ELISA tests are then performed to identify the clones which **produce the expected antibodies in a stable manner**.



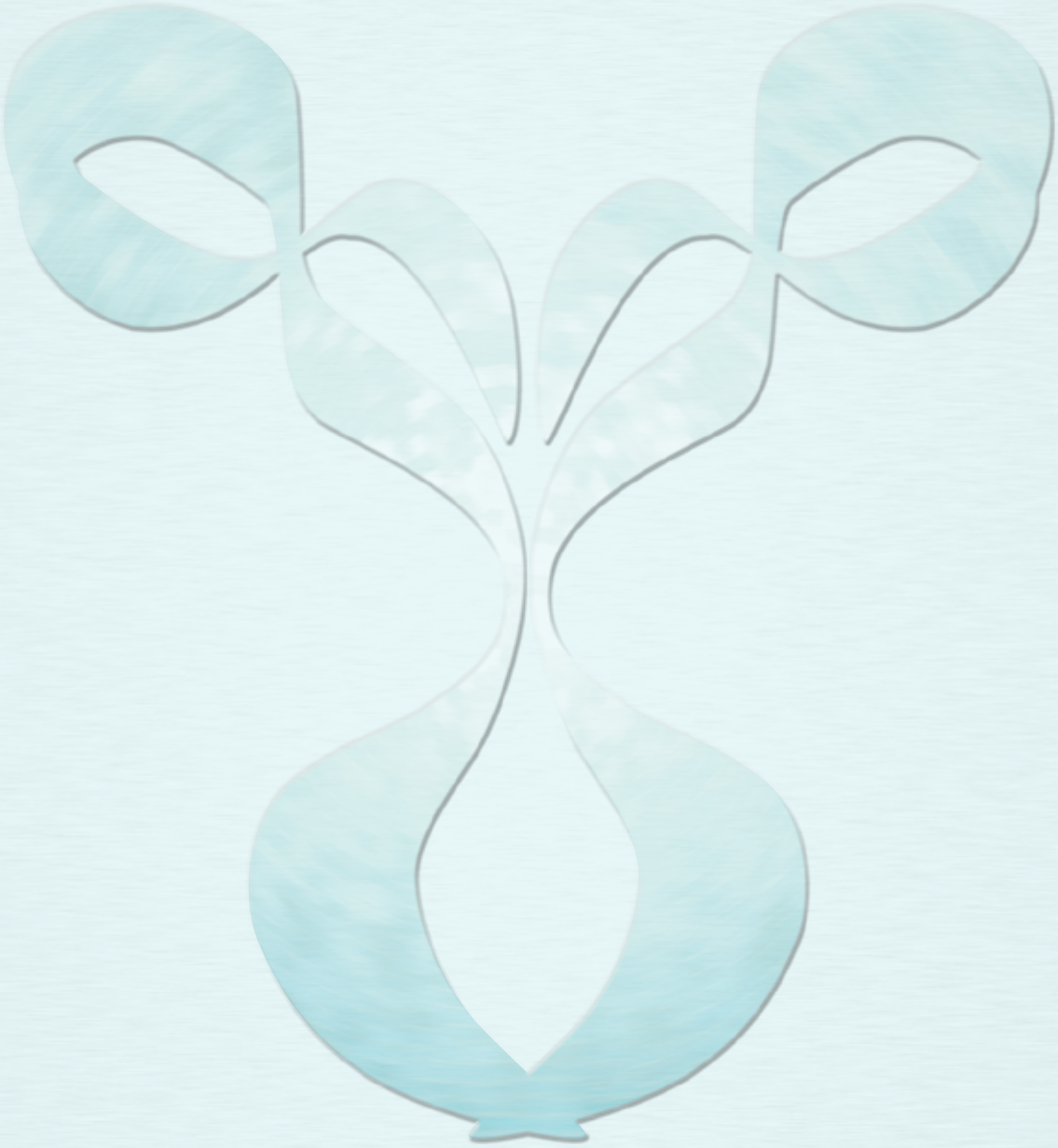
You receive:

- Supernatant from each confirmed stable positive clone to run tests in your specific conditions.
- The final clone frozen in a cryotube vial, based on both our results and yours.

Our guarantee:

- Cryotubes vials of the final clone you select are kept frozen in liquid nitrogen.

(2) Complete isotyping includes the determination of the class and subclass of the heavy chains as well as the isotype of the light chain.



covalab
R&D in Biotechnology

www.covalab.com

Covalab S.A.S. — 11 avenue Albert Einstein 69100 Villeurbanne — France
Phone: +33 (0) 437 654 230 — **Fax**: +33 (0) 437 289 416
E-mail: enquiries@covalab.com