MONOCLONAL ANTIBODIES

Expert in Antibody Engineering

From Research to Discovery

ISO 9001 : 2015 certified

covalab
R&D in Biotechnology

PROTOCOL OVERVIEW

1 IMMUNOGEN SELECTION

Protein
Microorganism
Peptide & PTM
Hapten
Other

2 IMMUNIZATION

4 mice or 2 rats
3-month protocol

ELISA tests on each serum
Follow-up of sera immunoreactivity over time

You choose the animal from which splenocytes will be extracted.

3 FUSION

Splenocytes extraction

Fusion of spleen cells with myeloma cell line

Culture in HAT selective medium

4 SCREENING

ELISA tests on growing cell hybridomas

Cell expansion from each positive well

ELISA tests to confirm hybridoma stability

Partial isotyping *(IgG or IgM)* and freezing

You choose one or several positive hybridomas for cloning.

5 CLONING

Choice of positive clones for cell expansion

ELISA tests to confirm antibody production stability

Complete isotyping *(isotype, class, subclass and light chain isotype)* and freezing

You choose the final clone from each selected hybridoma.

You can stop here or continue with in-vitro antibody production.

CUSTOM MONOCLONAL ANTIBODIES

During the immune reaction, antigen-processing cells activate quiescent B-cells into plasmocytes to secrete immunoglobulins directed against that antigen.

From the multiple plasmocytes clones involved in the immune reaction, each one can be isolated from the others by common cell culture techniques. Once isolated, this cell and its descendants produce identical immunoglobulins of the same subtype.

These immunoglobulins are then called monoclonal antibodies, and share the same affinity and specificity for one single epitope of the desired antigen.

COVALAB, YOUR PARTNER IN ANTIBODY ENGINEERING SINCE 1995

MADE IN FRANCE - ISO 9001 : 2015 CERTIFIED

Advantages

In vitro production

The most **reproductible** experiments

Very high **specificity** for their epitope

Reduced risks of unexpected cross-reactivity

Very **low background** staining

Large-scale production (up to miligrams in a week)

Drawbacks

High technical skills required that our engineers hold

Much more time production than polyclonal antibody (up to 3 time)

More expensive than polyclonal antibodies

Real issue? Prefer our polyclonal antibodies development !

IN-VITRO ANTIBODY PRODUCTION

We offer you a high quality hybridoma production whether you have a monoclonal antibody development project with Covalab or you already have an hybridoma.

Thanks to suitable cell culture materials and well-established procedures, we can achieve the production of *in vitro* monoclonal antibodies from milligram to gram scale really quickly without the hassle of handling animal.

DETAILED PROTOCOLE

1. IMMUNOGEN SELECTION

Protein

Microorganism
Inactivated virus
Inactivated yeast
Inactivated bacteria

Peptide PTM

Hapten
Carbohydrates
Chemicals & Toxins
Natural or modified nucleotides

Other
Cell extracts
Tissus extracts
Others *(please contact us)*

2. IMMUNIZATION

We offer you different protocol according to the hosts : 4 BALB/c mice or 2 Wistar rats.

4 BALB/c mice

Standard protocol
90 days
7 injections
4 test sera

OR

Exclusive Multisite protocol ®
94 days
12 injections
5 test sera

2 Wistar rats

Standard protocol

90 days

5 injections

3 test sera

All the experiments are carried out by experienced and authorised personnel according to H&S procedures, established in accordance to the French legislation. Our animal house is registered under the reference C21 464 04 EA.

YOU RECEIVE

- Sera to run tests in your conditions to choose the most suitable animal to perform the fusion step.

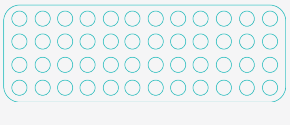
STORAGE & GUARANTEE

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

3. FUSION

Once the animal has been chosen according to both ELISA tests and your own results, we proceed to the isolation of its splenocytes.
These cells are subsequently fused with immortalised murine myeloma cells to generate hybridomas.

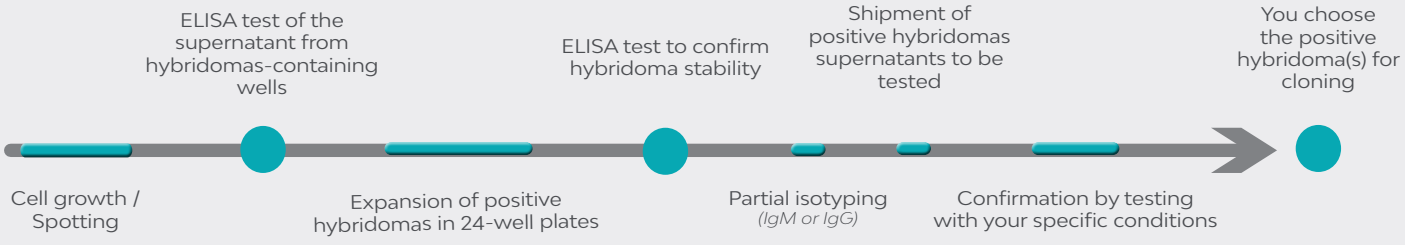
Fusion between splenocytes and myeloma cells in the presence of PEG



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

4. SCREENING

After several days of cell culture, the plates are screened to identify the presence of hybriodma cell lines. To check their antibody secretion, the corresponding culture supernatants are tested by ELISA method using the screening method developed previously. At the end of this step, the positive hybridomas are still made of multiple clones which must be isolated.



YOU RECEIVE

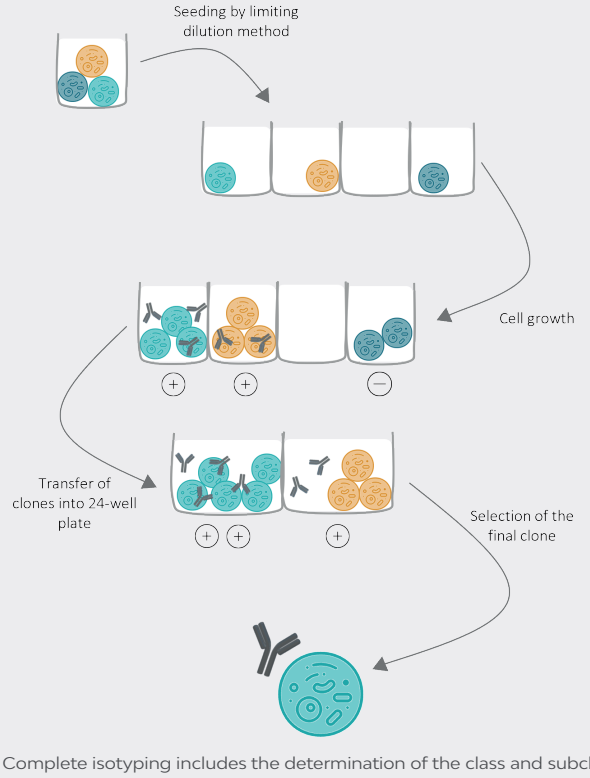
- Culture supernatant from each positive hybridoma to run tests in your conditions to choose one or several hybridomas to be cloned.

STORAGE & GUARANTEE

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

5. CLONING

The final step of the development procedure consists in seeding the previously 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

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

STORAGE & GUARANTEE

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

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

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