AGRO-BIO
The Proteomic & Immune Response

2 allée de la Chavannerie
45240 La Ferté Saint Aubin
France
Tel. 33 (0)2 38 64 83 50
Fax. 33 (0)2 38 64 83 59
www.agro-bio.com

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Protocol for the development of rat monoclonal antibodies

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Strengthened by its capacities of innovation recognized by the scientific community, Agro-Bio developed a new offer of service allowing the development of hybridomas by fusion of splenocytes of rat hyper-immunized with a rat myeloid cell line, as homologous partner.

This innovation allows generating a new register of monoclonal antibodies different from the classical register of mouse monoclonal antibodies. Rat monoclonal antibodies allow also reconsidering mouse as experimental model in the study of human pathologies. Thus, IgG2b of rat are tools for induction of cytotoxicity in human cells K.
For some antigens, the rat can present a good alternative to the mouse, by leading a better immunological response.

Furthermore, rat immunoglobulins present physical-chemical and biological properties different from those of the mouse. For example, the IgG1, IgG2a and IgG2b binds the human complement and the rabbit complement.

The success of rat monoclonal antibodies project is based on a perfect communication between Agro-Bio and the customer laboratory, from the beginning of the project.

Each file is treated in total confidentiality, which can be strengthened by the signature of an Agreement of specific confidentiality.

Standard hybridoma development is as follows (**minimum: 5 months**):

Initialization phase of the project:	- **Fill in the specifications sheet for the development of your monoclonal antibodies** and return it, - Agro-Bio will assess the feasibility of your program, - Upon request, a bibliography on your antigen or developed antibody can be achieved. Additional costs will be charged.
Phase I: Immunization **(9 weeks)**	Our standard protocol on 3 rats includes : - Immunization of animals according to a 63-day protocol. - Development of **ELISA method specifically developed** to follow the immune response of animals in your protocol, - Monitoring the immune response of immunized rats in order to determine the rat with the best serum titer, - Choice of rats for fusion.
Phase II: Fusion **(2 weeks)**	- Splenocytes from the best rat are fused with myeloid cells, - The first hybridomas are identified 10 days after the fusion.
Phase III: Primary screening **(4-5 weeks)**	- The culture supernatants from the identified wells are tested via the ELISA method previously developed, - The positive hybridomas are then amplified so as to undergo a second round of screening on their **specificity towards the antigen,** - A bank of 5 cryotubes is made.
Phase IV: Cloning **(2 weeks)**	In collaboration with the customer, the chosen hybridoma is **cloned** by successive dilutions.
Phase V: Secondary screening **(3-4 weeks)**	- A secondary screening on the obtained clones is done by ELISA, - The best positive clone is selected and **a bank of 10 cryotubes** is made.

The primary screening and the secondary of the development of rat monoclonal antibodies is realized by ELISA method. On the other hand, Agro-Bio advises the realization of the secondary screening in the final application of antibodies (with possibility of sending supernatants for control in parallel by the customer).
For any supplementary control, an estimate will be proposed.

Within two weeks following your order, you will receive a provisional timetable listing the various stages of your service, thus facilitating the communication and the follow-up of the program.

Included in our protocol:

- The purchase of animals, the quarantine period and the transfer to experiment,
- The guard of animals during the protocol,
- The injections and withdrawals of animals.

Not included in our protocol:

- The charges.

Cf. General terms of sale.

Agro-Bio is approved by the prefecture of Loir-et-Cher for experimentation on vertebrate animals.
Accreditation number: B 41-285-4