Custom Monoclonal Antibody Service

Mouse and Rat

A fast and flexible service according to your needs

Protein analysis cannot be imagined today without monoclonal antibodies which are mainly produced by mice. The use of mice as immunized animals, however, is posed to be a challenge in case the antibodies are intended to be raised against mouse proteins. Moreover, for some assays the use of antibodies of different species is essential. Therefore, we are now also offering rat antibodies using the well-established mouse antibody service.

Requirements and procedure

Phase I / Immunization
For immunization we require 1 mg of purified protein* or 3 mg of purified peptide. As a general procedure we immunize three Balb/c mice or two Lou/c rats with 90 to 150 µg peptide each. If required, we can switch to other mouse strains in the case the provided antigen is critical concerning its immunogenicity (e.g. mouse proteins or peptides).

Mice and rats are immunized over a period of 17 days every second day and one day before fusion. Depending on the antigen we can use different immune stimulating agents like Gerbu or incomplete Freund's Adjuvant combined with CpG oligo-nucleotides (A. M. Krieg, 2002; Annu. Rev. Immunol. 20: 709-760).
* if a Western blot screening is requested (cat. no. 2-2601-004), one additional mg protein is required.

Phase II / Fusion
After immunization, cells from knee lymph knots of all three mice or of two rats are prepared and fused with Ag8 myeloma cells in the presence of PEG 4000.

Phase III / Selection, ELISA screening
After completion of cell fusion the cells are plated on 24-well plates which results in 360 oligo clones each usually consisting of 5 - 10 clones. Cells are cultivated for 10 days in selective and conditioned Optimem medium selecting for fused cells only.

Within the next two days, the clones of interest have to be selected. All 360 oligo clone supernatants are screened by ELISA for antibody positive wells.

Phase IV / Western blot or immunofluorescence screening (optional)
For the next screening step we offer three options:

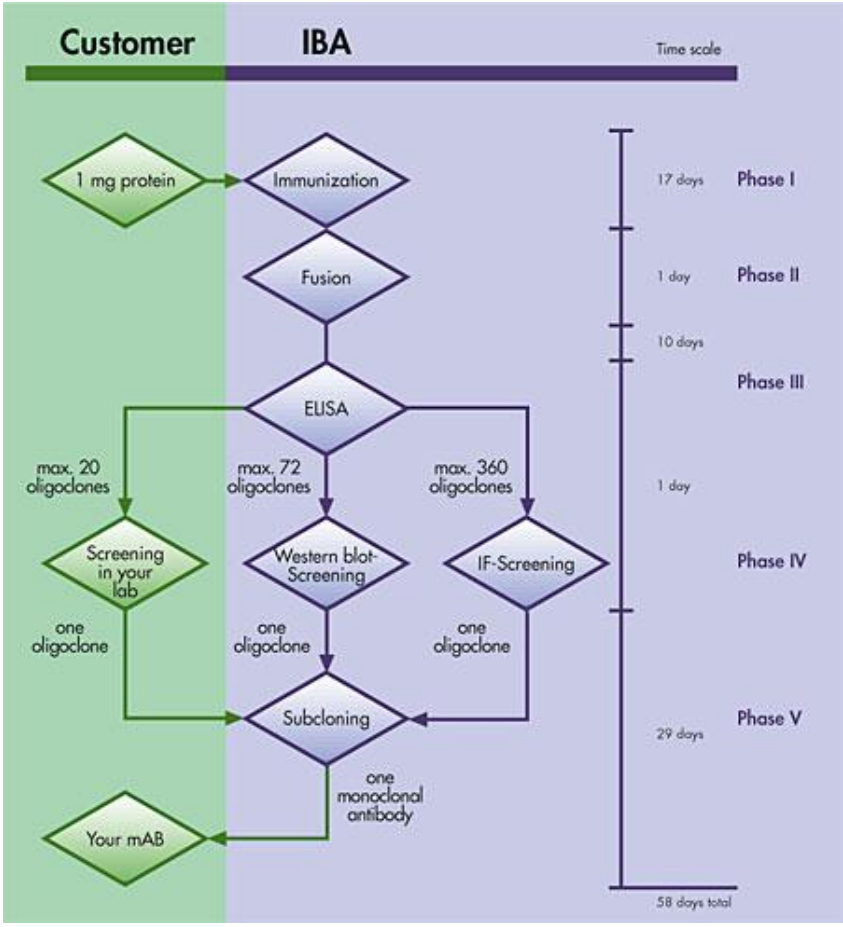
a. A maximum of 72 oligo clone supernatants are tested in Western blots.
b. A maximum of 360 oligo clone supernatants are screened by immunofluorescence against target cells provided by the customer (e.g. cells transfected with fluorescently labeled fusion protein).
c. A maximum of 20 oligo clone supernatants (5-10 ml) are shipped to the customer for screening under his own conditions.

Phase V /Subcloning and production
Usually screening is completed within a period of three weeks. The customer selects one (or more) oligo clone(s) which is (are) subsequently subcloned to monoclonality by the limiting dilution method. Isolated colonies are tested by ELISA, and positive ones are selected to be expanded for the next cycle of cloning.

Several cycles of cloning may be required in order to obtain a true, stable clone, which is subsequently used to produce 100 ml supernatant of the monoclonal antibody. Cells originating from the positive clones are frozen as back up. As part of our service, we are storing the clones until the next production.

Antibody production
Different production scales are available starting at 100 ml supernatant up to 100 mg purified antibody or more.

IKA also offer antibody production services from hybridoma cell lines provided by the customer. Please contact us for further information.



Standard protocol			
mAB generation	**description**	**mouse**	**rat**
		cat. no.	**cat. no.**
Phase I /Immunization	3 Balb/c mice or 2 Lou/c rats, 17 days immunization, pre-bleeding and immune serum on request	2-2601-001	2-2602-001
Phase II /Fusion	Preparation of lymphocytes, fusion with myeloma cell line	2-2601-002	2-2602-002
Phase III /Selection	Selection, ELISA screening	2-2601-003	2-2602-003
Phase IV /optional Western blot screening	Screening of max. 72 ELISA positive oligoclones in a Western blot against the antigen provided by the customer	2-2601-004	2-2602-004
Phase IV /optional IF	Screening of max. 360 oligoclones in an immunofluorescence assay against target cells provided by the customer	2-2601-005	2-2602-005
Phase V /Subcloning	Subcloning by limiting dilution, second ELISA screening, production of 100 ml supernatant of selected antibody	2-2601-006	2-2602-006