Development and characterisation of monoclonal antibodies.

We are able to immunise mice for monoclonal antibody production with any user-supplied protein or peptide antigen. We need only the antigen for immunisation and antibody screening (we are happy to discuss your specific requirements for screening antibodies that have the desired characteristics). To ensure timely and efficient monoclonal antibody production, we use 4 mice per antigen, with up to 5 injections per animal. In our standard protocol, immunisation of one mouse is performed using 50 μg of purified protein per injection per mouse. Therefore, we need 1 mg of purified protein for immunisation, plus an additional 250 μg for antibody screening. Less protein could be used if there is a problem with purification, but we would need to discuss this prior to starting the project. If you wish to use a peptide antigen, we need to be supplied with 2 mg of peptide coupled to KLH for immunisation and 1 mg of peptide coupled to BSA for screening of positive hybridomas. Please note that we can help with peptide design, synthesis and conjugation if needed. Please contact us to discuss your requirements.

Unlike many competitors, to increase efficiency of subsequent hybridoma testing and characterisation we screen for antibodies that recognise your specific target protein, rather than simply screening for immunoglobin production. When using a peptide-conjugate as immunogen, we also prefer to have purified or partially purified protein for secondary screening, because only 20 - 40% of antibody clones that recognise the peptide will recognise the target protein. To be as productive as possible in our work for you we recommend both types of screening.

Project proposal for development of monoclonal antibodies to one supplied antigen:

Development of monoclonal antibodies recognising ***one antigen*** (peptide or purified protein)

The protocol includes:

a) immunisation of 4 mice with your antigen
b) screening of polyclonal sera for recognition of antigen and selection of the best responder
c) fusion using splenocytes from one or two mice that show response; hybridoma production
d) screening of multiple individual hybridomas for production of Ig (dot-blot using rabbit anti-mouse specific Ig)
e) screening of Ig-secreting hybridomas for recognition of the target antigen (dot-blot using your antigen)
f) cloning and re-cloning of positive hybridoma cells
g) Ig subclass identification
h) master stock of hybridoma cell production and storage in liquid nitrogen for one year for free

i) production of 20 ml tissue culture supernatant of each hybridoma recognising the target antigen
j) TNT delivery of growing hybridoma cells and 20 ml of each positive supernatant

Payment is split into two parts:

a) Up-front payment to cover basic charges of animal costs, immunization, sera testing, fusion and first screening of fused hybridomas. We cannot take the risk of non-successful immunisation if the antigen is not immunogenic etc. We are not responsible for choice of antigen and its preparation, although we are happy to discuss this with you prior to initiating the project.

b) Final payment after fusion is successful and we have produced at least one hybridoma recognising the immunogen. This payment covers production of hybridomas and their secondary screening for reactivity to the antigen, two rounds of single cell cloning, frozen cell storage for 1 year and production of supernatant(s). The price includes delivery of hybridoma supernatants and growing cells via TNT.

Time schedule:

The time schedule is given in ranges as there could be delays due to weak immunogenicity of the antigen, or if hybridoma cells grow very slowly. The difference in time is generally only 2 or 3 weeks for the whole project.

Phase	Procedure	Description	Time
1	Immunisation	4 mice immunised using 10-50 μg of antigen (5 injections per mouse) and screened for response	8 - 12 weeks
2	Cell-fusion	Fusion of splenocytes and myeloma cells	Week 9 or week 13
3	Screening	Screening of hybridomas for production of Ig and recognition of specific antigen	Week 10 or 14
4	Propagation of hybridomas	Cryopreservation and production of cell bank	Week 12 or 16
5	Re-cloning	Recloning of all positive hybridoma cells and Ig (IgG1, IgG2a etc.) characterisation	Week 13 to 15
6	Expansion	Production of 20 ml of supernatant from each clone and preparation of cell bank for each cloned hybridoma	Week 14 to 16

Complete confidentiality is guaranteed and all the intellectual property and hybridoma usage belongs exclusively to the customer.

We keep the hybridomas for one year in our liquid nitrogen cell storage system. Further storage can be ordered for an additional cost per year for all hybridomas, including re-cloning every 3rd year.

Large-scale antibody production, purification, direct labelling etc. are also available on request.

We require a close link with the scientist ordering monoclonal antibody development. This contact is vital for the development of a successful screening strategy to provide antibodies with the user-defined operational characteristics, which is the critical aspect for successful production of monoclonal antibodies. We provide ongoing updates throughout the procedure to aid joint decision making with the customer, and we expect timely responses to any requests from us regarding user-generated data on antibody clones.

<u>Additional services:</u>

1) ***Large scale antibody production, purification and characterisation***
    a) purification of 2-5 mg of Ig includes (i) large scale antibody production in serum free media, (ii) protein A or L purification of Ig, (iii) dialysis against PBS or other required buffer, (iv) measurement of protein concentration, (v) analysis of Ig purity using gel electrophoresis.
    b) delivery of purified antibodies via 24hr TNT service
2) ***Direct labelling of purified Ig with (i) biotin, (ii) horseradish peroxidase, (iii) alkaline phosphatase, (iv) other potential groups of labels***
    a) production and purification of 2-5 mg of Ig in serum free media
    b) labelling of 1-3 mg of purified Ig using SH group
    c) delivery via 24 hr TNT service

We will deliver as many hybridoma supernatants as are produced from the fusion (generally 2-10 hybridomas) and we will clone any hybridoma that the customer indicates as useful for their work. We will produce two clones for each hybridoma. Clone 1.1 is used for most work, plus clone 1.2 that is a back-up hybridoma in case the first clone is lost, grows slowly, loses activity, or if the customer sees it as a better reagent than clone 1.1. These two clones are generated using two single cell-derived clones isolated from the same original hybridoma.