

Hybridoma Production



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Phase I: Immunization
(Price: € 900,-; time frame: approximately 60 days)

Detailed protocol

- The monoclonal antibody protocol starts with the primary immunization of three mice with antigen emulsified with Complete Freund's Adjuvant (CFA)
- After 14-21 days, the mice receive a second injection of the antigen with Incomplete Freund's Adjuvant (IFA).
- After 28-42 days, the mice receive a third injection of the antigen with (IFA)
- After 42-60 days, the mice receive a fourth injection of the antigen with (IFA)
- The first blood sample is obtained 7 days after the third injection
- The polyclonal serum samples are screened by ELISA to determine the comparative titres of antigen-specific antibody. (Alternatively, the serum samples can be shipped to the client^x for screening with a different assay, or MQR can perform customized assays according to the clients' specifications^x)
- The mouse with the best response receives a pre-fusion boost and is sacrificed for a fusion. Alternatively another round of immunizations and a second bleeds^x can be performed on all mice. Following a second serum assay the client decides whether the best responder mouse is selected for a fusion, or the entire cage of mice is terminated.

Phase II:

A - Option 1) Direct Fusion plus automated cell screening & picking with ClonepixFL
(Price: € 1950,-; time frame: approximately 3-4 weeks, using one screening parameter)

Detailed protocol

- After the pre-fusion boost, the best responder mouse is anesthetized and terminated by exsanguination and the lymphocytes are isolated from the spleen.
- The lymphocytes are mixed with NS1 or sp2/0 myeloma cells in fusion media and fused using an MQR electro-fusion protocol.
- Since MQR's fusion efficiencies are very high MQR will store, at the clients disposal, remaining lymphocytes derived from the animal used for the fusion experiment until termination of the project. For fusion of NS1 myeloma cells MQR uses $1-2 \times 10^7$ lymphocytes, for sp2/0 myeloma cells $5-10 \times 10^7$ lymphocytes are used.
- Immediately following the fusion event, hybridoma selection is performed in semi solid media containing HAT.
- Within 10-14 days all surviving hybridomas are screened using our **ClonepixFL** automatic colony picking device allowing for fast isolation of individual antigen specific primary hybridoma clones, in this screening we can simultaneous screen for two antigens, and/or specific isotype producing clones, and/or high level antibody producing clones.
- In our standard protocol using the colony picking procedure, 10 primary cultures are expanded for cryopreservation, and single frozen copies of each are shipped to the client along with the 4 ml of the antibody-containing culture media the cells were growing in at the time of the freezing.

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A- Option 2) B cell selection and culture using automated cell screening & picking ClonepixFL
(Price: € 4500,-; time frame: approximately 5-6 weeks, using one screening parameter)
This option is most suitable for difficult targets (i.e. protein modification specific antibodies)

Detailed protocol

- After the pre-fusion boost, the best responder mouse is anesthetized and terminated by exsanguination and the lymphocytes are isolated from the spleen.
- Subsequently, the lymphocytes are selected on antigen using MQR protocols.
- Selected B-cells are cultured for 10 days, and screened for antigen specific colonies. 10 best B-cell colonies are subjected to mini-electrofusion using NS1 myeloma cells.
- Immediately following the fusion event, hybridoma selection is performed in semi solid media containing HAT.
- Within 10-14 days all surviving hybridomas are screened by the MQR **ClonepixFL** automated colony picking procedure in order to isolate the primary hybridoma cultures that secrete monoclonal antibody specific for the client's antigen, (optional simultaneous screening for isotype and production levels can be performed^x).
- In the MQR standard protocol, 5 primary cultures are expanded for cryopreservation, and single frozen copies of each are shipped to the client along with the 4 ml of the antibody-containing culture media the cells were growing in at the time of the freezing.

B- Subcloning and Antibody Characterization
(Price: € 2250,-; time frame: approximately 4 weeks)

Detailed protocol

Artificially fused cells are initially genetically unstable. Such cells might expel a chromosome carrying a gene necessary for antibody production but retain their ability to divide, and can potentially overgrow antibody secreting cells.

- The 5 best primary cultures as determined in Phase II are subcloned twice in semi solid media using our **ClonepixFL** automatic colony picking device.
- (Optional^x) The client may decide whether during this subcloning clones are analysed for isotype and/or antibody production levels. Clones with highest production level and/or specific isotype can be picked.
- Each subcloned set is expanded and frozen into four vials each.
- At the completion of Phase III, for each primary culture subcloned the client receives 2 vials frozen cells, 10 ml culture media the clones were growing in at the time they were frozen. 2 vials frozen cells will be stored at MQR for 6 months as a back up for the client.

Subcloning of additional primary cultures will be charged at € 450,- per clone.

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Phase III: Bioreactor CL1000 Integra Antibody Production (Price: € 2000,-[#] time frame: apprixomately 6-8 weeks)

MQR utilizes in vitro methods of antibody production as compared to the ascites fluid method, which needlessly destroys animals.

At MQR, an Integra CL1000 bioreactor is run for 4 weeks. Clients can expect a yield between 50 and 200 mg of their monoclonal antibody per run. Due to variable secretion rates of hybridomas, the actual amount of antibody generated is dependent on the unique characteristics of each hybridoma. Additional cost will apply for purification for the antibody.

General Conditions

- * B-cell selection and culture procedure prices are for research applications only. For Diagnostic or therapeutic applications additional conditions will apply.
- # Prices depend on culture medium required and actual running time. Standard production run is 4 weeks. 2-4 weeks start-up time.
- x indicates that additional cost will apply.

Prices are valid till 01-01-2009