

mAb Development Program

DEVELOPMENT AND SCREENING OF HYBRIDOMA FOR THE PRODUCTION OF ANTIBODIES TO THE ANTIGEN(S) THROUGH IMMUNIZATION IN MICE.

Polo GGB will generate hybridoma lines producing mAb which are specific binding to Protein X in microarray and provide milestone samples (antiserum and supernatants) for customer in house screening.

STAGE I (12 weeks): IMMUNIZATION OF MICE, SERUM COLLECTION AND IMMUNE RESPONSE MONITORING

Procedure		Schedule (weeks)	Immunization type
Step 1	Pre-Immune Bleed	-1	
Step 2	Primary Immunization	0	antigen(s) - CFA* adjuvant
Step 3	1st Boost	2	antigen(s) - IFA** adjuvant
Step 4	2nd Boost	4	antigen(s) - PBS
Step 5	Test Bleed 1	5	
Step 6	3rd Boost	6	antigen(s) - PBS
Step 7	Test Bleed 2	7	
Step 8	Final Boost	12	antigen(s) - PBS
Step 9	Cell Fusion	Final boost + 4 days	

*CFA: Complete Freund's Adjuvant ---** IFA: Incomplete Freund's Adjuvant

Test bleeding of immunized mice: immunized animals will be screened by microarray technology. The immune response will be compared to the pre-immunization bleed. After eight weeks from the start of immunization process, Polo GGB (if requested) will deliver antiserum (15µl/animal) to the customer for in house assay

STAGE II (3 - 4 weeks): FUSION HYBRIDOMA DEVELOPMENT AND ANTIBODY SCREENING SERVICES

Spleens from the selected animal will be utilized to perform the cell fusion. Each fusion will be plated onto 96-well plates and supernatants will be screened by microarray technology.

STAGE III (3 - 4 weeks): HYBRIDOMA SUBCLONING

Positive hybridomas will be sub-cloned by limiting dilution and supernatants will be screened a second time to confirm that the expanded fusion products are stable and actively secreting antibody still.

Two positive parental clones will be expanded and cryo-conserved.

Polo GGB, if requested, will deliver 1-2 ml supernatants to the customer for in-house assay.

STAGE IV: ISOTYPE DETERMINATION BY E.L.I.S.A ASSAY

STAGE V (2 - 3 weeks): PRODUCTION AND PURIFICATION OF MAB BY PROTEIN G CHROMATOGRAPHY METHOD

STAGE VI (1 week): MAB PURITY TEST BY SDS-PAGE AND MAB REACTIVITY CONFIRMATION BY MICROARRAY AND WESTERN BLOTTING IMMUNOASSAYS

Service Specifications

Service includes five (5) years: Storing and maintaining of cell lines for 5 years. After this period, Polo GGB will eliminate every biological material. Upon request and with additional costs, the cell lines can be maintained.

Total Time Project: 4-6 months for mouse mAb production.

Clone characterization: mAb purity evaluated by SDS-PAGE. Reactivity and Specificity of the mouse monoclonal and chimeric antibodies determined by Microarray and Western Blotting assays.

Quality control: Laboratory Certificate of Analysis.

Reagent/Information needed to supply: Antigens required for immunization of animals and screening hybridomas (minimum 1mg/each antigen) and their characteristics. *Note: Polo GGB reserves the possibility to request an additional quantity of 0.5mg for each antigen to be use for screening and characterization assays.*

Shipment: Monoclonal and Chimeric Antibodies will be shipped to client facility with an adequate package to guarantee a temperature (°C) comprises between $\pm 4^{\circ}\text{C} <T^{\circ}> \pm 8^{\circ}\text{C}$.

Reagent Stability: 1 year at +4°C. 10 years at -20°C in glycerol.

Please, store the product at +4°C until opened, then aliquot and freeze at -20°C. Avoid repeated freezing and thawing.

Use of the newly produced clones: The client will have sole and unrestricted ownership of the newly developed reagents and the scientific data, commercial rights, IP.

NOTE of the PROVIDER: Assays and screening methods will be performed using Polo GGB technologies. The screening method is based on microarray immunoassay. The array will contain the antigen(s) in several concentrations, positive and negative controls.