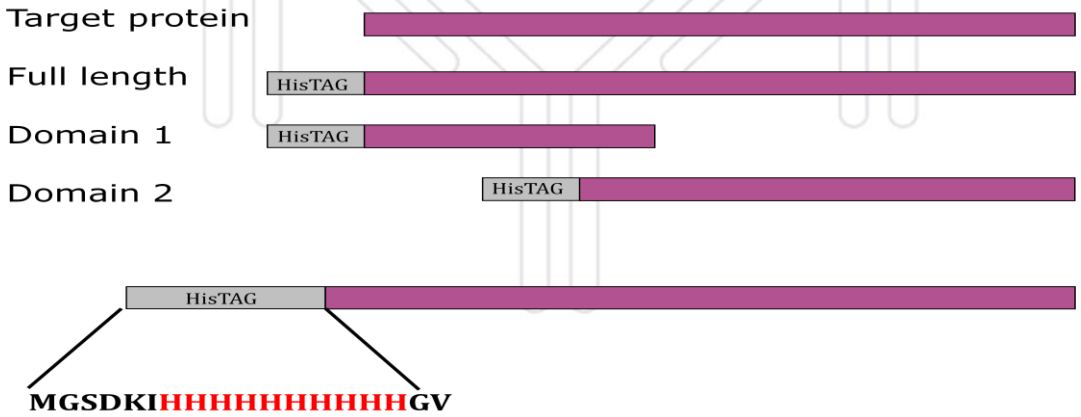
# CUSTOM MONOCLONAL ANTIBODIES AGAINST PROTEINS AND PEPTIDES *(mouse)*

## ANTIGEN

Approx. 3 mg of protein antigen are necessary for immunization and analysis.
Primm can provide custom antigens as recombinant protein or conjugated synthetic peptide in case the protein antigen is not available.

### Production of recombinant protein

Primm offers a very competitive service based on an optimized fast and efficient technology to produce recombinant protein for immunization. Starting from the nucleotide gene sequence or from a gene ID number/name related to public genomic databases (human, mouse, rat origin), Primm will proceed to the cloning of the cDNA sequence in proprietary expression vector. If the gene target is from unusual organism or species and a reliable RNA source is not available, the customer may be requested to supply the gene. After complete DNA sequencing of the expression clone, our team will proceed with production in *E. coli* of the His-tag fusion protein; purification is performed as "standard grade" in denaturing buffer, which typically yields good quality for immunization.
Depending on the protein size and features, full length protein and 2/3 domains (up to 300 aa) will be chosen for expression. Primm will attempt production and purification of all selected domains; the best one, in terms of purity and yield, will be used for immunization. For the selection of recombinant domains, our staff will take into consideration specific needs and recommendation from customer.




### Synthesis of peptide and conjugation

- Synthesis of about 10 mg of peptide, purity >80%
- Conjugation of 3-4 mg of peptide to carrier protein, Ovalbumin, KLH or other carrier protein requested by customer, to be used for immunization
- Conjugation of 1-2 mg of peptide to a different carrier protein to be used for ELISA screening to discriminate between anti-peptide and anti-carrier antibodies

# ANTIBODY

The development and production of monoclonal antibodies goes through different phases:

## Immunization Phase (1.5 month)

Immunization of 4 BALB/c mice with the antigen. Injections of 20-30 µg + CFA (Complete Freund Adjuvant) are done at $T_0$, while 10-15 µg + IFA (Incomplete FA) are done at $T_{21}$, $T_{28}$ (days) subcutaneously.
ELISA testing of the immune response at $T_{35}$ and selection of the animals for subsequent spleen cell fusion.
Deliverables: ELISA Tests.
Analytical bleed, if requested (20-50 µl), will be provided at additional charge. Primm will keep the mice/rats alive for 3/4 weeks (1 additional boost is included) to allow customer decide which 2 mice will be selected for spleen fusion.

## MAB Development Phase (3 months)

Spleenectomy, fusion of spleenocytes with myeloma cells and screening of mother hybridoma clones.
Highly specific antibodies can be obtained by fusing tumor cells with B cells from the spleen of immunized animals (generally mice, but we can also use rats or hamsters). The fusion allows to maintain the characteristics of both cellular type involved: the B cell ability to produce antibodies and the tumor cell line immortality.
Cells derived from fusion is then divided in approx. 1,000 wells of 96-well plates, each carrying 200 µl of culture. Hybrid cells (hybridoma) are specifically isolated by selection in media carrying HAT which prevents the growth of non fused cells.
Cells are grown for 2 weeks and 50 µl are taken for ELISA testing.
ELISA-positive clones are expanded from 96 to 24 well plates and when they become 80% confluent, supernatants are tested again to confirm positivity.
ELISA-positive clones are then expanded from 24 to 6 well plates. Again, when they are 80% confluent, a final ELISA test is performed to verify positive recognition of the antigen.
Cloning and subcloning of positive clones. Monoclonal antibodies are secreted by the progeny of a homogeneous cell population that can produce only a single immunoglobulin. The cloning by limiting dilution is required to ensure that poly-specificity is avoided and any risk of overgrowth by non-producing cells is minimized.
Hybridoma cells are dispensed in 96 well-plates to a concentration of 1 cell/well and 0.3 cell /well. Accordingly, a homogeneous cellular population can be obtained starting from a heterogeneous mother clone.
The cell expansion procedure is equivalent to what is done for the isolation of initial clones. Cells are expanded from 96 to 24 to 6 well plates, testing ELISA positivity at each step). Positive clones are then expanded in T25 flasks and supernatants immunoaffinity purified. 2 vials containing $10^6$ cells are stored together with puirifed supernatants.

**Primm will deliver at least 5 final single clones. Cells are stored and transferred to customer together with available purified MABs.**

**It's important to underline that the hybridoma clones could produce either IgG or IgM. Primm strategy is to select IgG clones (using as secondary antibody a Goat anti-mouse IgG), upon customer request Primm can select both IgM and IgG (using as secondary antibody a Goat anti-mouse polyvalent immunoglobulins).**

## Production and Purification Phase, Optional (1-1.5 months)

Production and purification of Monoclonal Antibody in "cell culture"

Customer may request our service for the production and purification of small or large quantities of Monoclonal Antibody starting from a final clones selected at the end of Phase 3.
Small scale production **(0.5-2 mg)** is performed at either 50 or 200 ml of culture.
Antibody Subisotyping testing is performed to define the procedure to be used for antibody purification, Protein-A Sepharose for IgG and Hydroxyapatite for IgM.

## Hybridoma clone storage

Upon delivery of final hybridoma clones to customer, Primm will start a free service for the storage of 2 vials of each clone for a period of 3 months to allow customer to expand cells and establish his own back up. Following the free 3-month period, Primm offers a storage service for 6 months or multiples. If no additional storage service is requested by customers, Primm aliquots will be eliminated or delivered to the customer. Shipping fees will be charged.