



CUSTOM RAT HYBRIDOMA SERVICE (Regular Peptides or Protein Immunogens)

AnaSpec Inc. guarantees up to three (3) ELISA positive hybridoma clones for each custom hybridoma project.

We emphasize on (1) strong commitment towards each project, (2) seamless customer communication, (3) flexible protocol and schedule based on customers' needs, (4) shorter immunization time frame, and (5) on time delivery.

<p>Phase I: Animal immunization: Immunogen design (Complimentary) Peptide synthesis (20 mg, up to 20 mers with 80% purity) (optional) Or customer provided protein immunogen (>5 mg with >95% purity) Peptide-carrier protein (KLH/BSA) conjugation (optional) Immunization and ELISA screening</p> <p>Phase II: Cell fusion and parental clone screening</p> <p>Phase III: Monoclonal selection and cryogenic vial preservation</p>
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<p>Optional:</p> <p>Phase IV: Large scale monoclonal antibody production: In vitro cell culture (Bioreactor) (up to 50 ml) Monoclonal antibody purification (either protein G or neutral pH method)</p>

Additional screening services for antibody functionality:

Dual ELISA, subtracting ELISA, capture ELISA and/or detection ELISA	Please inquire
Western blot, dot blot, immunoprecipitation, and/or immunohistochemistry etc	Please inquire

Phase I: Animal immunization (6-8 weeks).

- Peptide and carrier protein(s) conjugation if required.
- Multiple immunizations of rats (**n = 3**).
- Selection of animals based on antiserum ELISA titer and functionality prior to the final boost and cell fusion in Phase II.

Phase II: Cell fusion and parental clone screening (3-4 weeks).

- Phase-I selected animal(s) will be given a final boost for cell fusion.
- Fused cell clones in 96 wells are initially screened by ELISA.
- Potential parental clones (up to 24 clones) will be expanded into 24-well plates to harvest cell culture supernatant for further ELISA screening.
- Shipment of up to 12 positive parental clone supernatants (0.5-1 ml/per parental clone) to customers for further parental clones screening.
- **Customer must give feedback within 1 week to ensure viability of clones.**

Phase III: Monoclonal selection and cryogenic vial preservation (3-4 weeks).

- Monoclonal selection of the best 1-3 clone(s) will be performed.
- Subclone(s) with high specificity and antibody titer will be expanded and cryopreserved.
- The cryopreserved vials (3 vials/each clone) and the culture supernatant (10 ml/each clone) will be shipped to customers.
- Isotyping of the monoclonal clones will be performed.



Phase IV: Large scale antibody production (4-6wks) (Optional)

- In vitro Bioreactor antibody production (up to 50 ml of supernatant for each clone).
- Monoclonal antibody purification from culture supernatant or ascites using either protein G or neutral pH method. ELISA and SDS-PAGE will be performed to ensure the quality of the purified monoclonal antibody.

Hybridoma Development Terms and Conditions:

1. The charge for peptide synthesis/purification is not refundable once the project is initiated.
2. The charge for Phase-I is not refundable once the animal immunization is started.
3. If Phase-II Cell Fusion results in no ELISA positive clone, we will repeat the phase-II once at no charge, but Phase-I will not be refunded.
4. Additional animal Phase-II Cell Fusion service is available at extra cost.
5. Additional potential parental clones expansion (to 24-well plate) can be provided at extra cost.
6. Additional parental clone Phase-III subcloning service is available at extra cost.
7. Additional monoclonal clone(s) (beyond 3 included in packages) can be provided at extra cost.
8. We cannot guarantee the success of projects where customers provide the peptide immunogens.

IMPORTANT NOTE: AnaSpec will store the parental clones and subclones for 6 months only. Storage fee applies if customers choose to keep the clones for more than 6 months at AnaSpec.