
1. PURPOSE

This Standard Operating Procedure (SOP) describes the procedures for producing polyclonal antibodies in rats. The rat can be used when antibodies of restricted specificity to mouse proteins are required.

2. RESPONSIBILITY

Principal investigator (PI) and veterinary care staff.

3. MATERIALS

- 3.1. Freund's Complete Adjuvant
- 3.2. Freund's Incomplete Adjuvant
- 3.3. Syringes and needles
- 3.4. Medical records
- 3.5. Acepromazine
- 3.6. Blood collection material
- 3.7. Isoflurane

4. PREPARATION OF IMMUNOGEN

- 4.1. The immunogen must be:
 - 4.1.1. Non-toxic
 - 4.1.2. Sterile
 - 4.1.3. Free of pyrogens
 - 4.1.4. pH within physiological limits
 - 4.1.5. Easily passed through a 21G needle

NOTE: Proteins in polyacrylamide gel may cause adverse reaction at the site of injection. Use another method of purification or a dilution when possible.
- 4.2. Prepare 4 samples of the immunogen (labeled 1-4), each consisting of 200-500 micrograms of antigen in sterile PBS in a volume of 0.1 ml (per rat).

5. PROCEDURES

- 5.1. Adjuvant:
 - 5.1.1. Use an adjuvant to increase the immunological response to poor antigens.

NOTE: When used with a strong antigen, the adjuvant may induce an overt local inflammatory response.
 - 5.1.2. Use Freund's Incomplete Adjuvant (FIA) and Freund's Complete Adjuvant (FCA). In case of an overt reaction, other adjuvants are available for use.
 - 5.1.3. Administer FCA ONLY ONCE for the primary injection. Do not repeat. Use only FCA with a concentration of 0.5mg/ml of mycobacteria or less.
 - 5.1.4. Use FIA for all secondary immunizations.
 - 5.1.5. Combine the antigen and the adjuvant using two syringes and locking connector (e.g., 3-way stopcock) and emulsify until it no longer separates.

- 5.2. Recordkeeping:
 - 5.2.1. Record the following information in the medical record of the animal.
 - 5.2.1.1. Name of immunogen
 - 5.2.1.2. Adjuvant used
 - 5.2.1.3. Route of administration
 - 5.2.1.4. Site(s) of injection
 - 5.2.1.5. Volume injected
 - 5.2.1.6. Date of injection
 - 5.2.2. Record blood collection volume and site as well as body weight, general condition, and appearance of the injection sites.
- 5.3. Animal selection:
 - 5.3.1. Use young (approximately 8 weeks of age), adult female Sprague-Dawley rats whenever possible.
 - 5.3.2. Allow a minimum of 7 days of acclimation after the arrival of the animals.
 - 5.3.3. Identify animals using ear punches.
- 5.4. Pre-immune blood sample:
 - 5.4.1. Sedate the animal by administering intramuscularly acepromazine 0.5-1.0 mg/kg.
 - 5.4.2. Collect 0.2 to 0.5 mL of blood from the saphenous vein.
 - 5.4.3. Centrifuge and freeze serum at -20°C.
- 5.5. Primary immunization:
 - 5.5.1. Combine the 0.1 mL antigen sample with 0.1 mL of FCA adjuvant.
 - 5.5.2. Inject the 0.2 mL sample subcutaneously into 4 sites, 0.05 mL per site, bilaterally along the thoracic-lumbar region of the spine. Injection sites must be sufficiently distant to prevent coalescence of the local inflammatory response.
 - 5.5.3. Do not contaminate the needle track with resulting intradermic or intramuscular deposition of the mixture. Before removing the needle, withdraw on plunger slightly to prevent the leakage of adjuvant into the dermal layer.
- 5.6. Wait the 3-to-4-week period necessary to build up a primary immunological response.
- 5.7. Secondary immunization:
 - 5.7.1. DO NOT REPEAT FREUND'S COMPLETE ADJUVANT (FCA). If FCA was used in primary immunization, use Freund's Incomplete Adjuvant (FIA), or another adjuvant.
 - 5.7.2. Give booster injections in the vicinity of the initial sites as long as there is no indication of inflammatory reaction from the initial injection.
 - 5.7.3. Proceed as indicated for the primary immunization in section 5.5.
- 5.8. Titer determination:
 - 5.8.1. Collect a 0.2 to 0.5 mL blood sample from the saphenous vein 3 to 4 weeks after secondary immunization as in section 5.4.
- 5.9. Repeat secondary immunization and titer determination every 3 to 4 weeks. In most cases, the antibody titer reaches an acceptable level after two boosters.
- 5.10. Animal monitoring:
 - 5.10.1. Observe animals for a minimum of 15 minutes post-injection for any abnormal reactions.
 - 5.10.2. Observe the animals daily for responses at the injection sites in particular and for overall health or distress in general.
- 5.11. If the titer is sufficient, euthanize the animal by exsanguination under general anesthesia or following euthanasia using CO₂:
 - 5.11.1. Deeply anesthetize the rats using isoflurane or euthanize rats using CO₂. Refer to SOPs 111 and 301.
 - 5.11.2. Exsanguinate via cardiac puncture.

5.12. Euthanize the animal if titer is still insufficient 6 months after initial immunization, or request authorization from veterinarian to pursue immunization.

SOP REVISION HISTORY

DATE	NEW VERSION
2022.01.13	3.7. Chlorhexidine
2022.01.13	4.1. The PI must prepare an immunogen that is must be :
2022.01.13	4.2. The PI must provide Prepare 4 samples of the immunogen (labeled 1-4), each consisting of 200-500 micrograms of antigen in sterile PBS in a volume of 0.1 ml (per rat).
2022.01.13	5.5.2. Clean the injection site with chlorhexidine.
2022.03.10	Polyclonal antibody production is provided as a fee for service through the Comparative Medicine and Animal Resources Centre (CMARC).