

**1. PURPOSE**

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This Standard Operating Procedure (SOP) describes the procedures for producing polyclonal antibodies.

Polyclonal antibody production is provided as a fee-for service through the Comparative Medicine and Animal Resources Centre (CMARC).

**2. RESPONSIBILITY**

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Principal investigator (PI) and veterinary care staff.

**3. MATERIALS**

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- 3.1. Freund's Complete Adjuvant
- 3.2. Freund's Incomplete Adjuvant
- 3.3. Syringes and needles
- 3.4. Microchip identification system
- 3.5. Medical records
- 3.6. Acepromazine
- 3.7. Butorphanol
- 3.8. EMLA cream
- 3.9. Blood collection material
- 3.10. Chlorhexidine
- 3.11. Ketamine
- 3.12. Xylazine
- 3.13. Glycopyrrolate

**4. PREPARATION OF IMMUNOGEN**

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4.1. The PI must prepare an immunogen that is:

- 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 20G 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. The PI must provide 4 samples of the immunogen (labeled 1-4), each consisting of 200-500micrograms of antigen in sterile PBS in a volume of 500µL (per rabbit).

**5. PROCEDURES**

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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.
- 5.1.1. 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, adult female Specific Pathogen Free rabbits (about 2 to 2.5kg) whenever possible.
  - 5.3.2. Allow a minimum of 7 days of acclimation after the arrival of the animals.
- 5.4. Pre-immune blood sample:
  - 5.4.1. Tranquilize rabbits by injecting intramuscularly (can be mixed in the same syringe):
    - 5.4.1.1. Acepromazine (0.5mg/kg)
    - 5.4.1.2. Butorphanol (0.2 mg/kg) or buprenorphine (0.2 mg/kg),
  - 5.4.2. Apply EMLA cream over the ear on the blood collection site 15 minutes before puncture.
  - 5.4.3. Collect 5 mL of blood from the ear artery. Centrifuge and freeze serum at -20°C.
- 5.5. Identify animals using microchips while still sedated.
- 5.6. Primary immunization:
  - 5.6.1. Combine the 0.5 mL antigen sample with 0.5 mL of FCA adjuvant and emulsify as in section 5.1.1.
  - 5.6.2. Rinse the injection site(s) with chlorhexidine.

**Note:** shaving of the injection site is not recommended as it can lead to increased aggression in group-housed rabbits.
  - 5.6.3. Inject the 1 mL sample subcutaneously into 10 sites, 0.1 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.
  - 5.6.4. 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.
- 5.7. Wait the 3 to 4 week period necessary to build up a primary immunological response.
- 5.8. Secondary immunization:
  - 5.8.1. DO NOT REPEAT FREUND'S COMPLETE ADJUVANT (FCA). If FCA was used in primary immunization, use Freund's Incomplete Adjuvant (FIA), or another adjuvant.
  - 5.8.2. Give booster injections in the vicinity of the initial sites as long as there is no indication of inflammatory reaction from the initial injection.
  - 5.8.3. Proceed as indicated for the primary immunization in section 5.6.

- 5.9. Titer determination:
  - 5.9.1. Collect a 8.5mL blood sample 3 to 4 weeks after secondary immunization as in section 5.4.
- 5.10. Repeat secondary immunization and titer determination every 3 to 4 weeks. In most cases, the antibody titer reaches an acceptable level after two boosters.
- 5.11. Animal monitoring:
  - 5.11.1. Observe animals for a minimum of 15 minutes post-injection for any abnormal reactions.
  - 5.11.2. Observe the animals daily for responses at the injection sites in particular and for overall health or distress in general.
- 5.12. If the titer is sufficient, proceed with one of the following:
  - 5.12.1. Euthanize the animal by exsanguination under general anesthesia:
    - 5.12.1.3. Inject glycopyrrolate (0.1mg/kg), acepromazine (0.5mg/kg) and butorpahol (0.2 mg/kg) subcutaneously. Wait 15 to 20 minutes.
    - 5.12.1.4. Inject xylazine (5mg/kg) intramuscularly. .
    - 5.12.1.5. Inject ketamine (20-35mg/kg) intramuscularly in a different muscle.
    - 5.12.1.6. Exsanguinate via cardiac puncture.
  - 5.12.2. Collect 8.5mL/kg of blood every 4 weeks. Do not exceed 6 months of total duration since initial immunization or request authorization from the veterinarian.
- 5.13. Euthanize the animal if titer is still insufficient 6 months after initial immunization, or request authorization from veterinarian to pursue immunization.