

POLYCLONAL ANTIBODY PRODUCTION - RABBITS

1. PURPOSE

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

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. Microchip identification system
- 3.5. Medical records
- 3.6. Acepromazine
- 3.7. Butorphanol
- 3.8. Dexmedetomidine
- 3.9. Atipamezole
- 3.10. EMLA cream
- 3.11. Blood collection material
- 3.12. Chlorhexidine
- 3.13. Ketamine
- 3.14. Xylazine
- 3.15. Gas anesthesia machine (calibrated within the last 12 months) with anesthesia mask and adequate gas scavenging system or filter
- 3.16. 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 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. Prepare 4 samples of the immunogen (labeled 1-4), each consisting of 200-500 micrograms of antigen in sterile PBS in a volume of 500 μ L (per rabbit).

5. PROCEDURES

5.1. Adjuvant:

- 5.1.1. Use an adjuvant to increase the immunological response to poor antigens.
- 5.1.2. **NOTE:** When used with a strong antigen, the adjuvant may induce an overt local inflammatory response. 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 0.5 mg/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-

- 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 an 8.5 mL 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.
- 5.12. If the titer is sufficient, proceed with one of the following:
 - 5.12.1. Euthanize the animal by exsanguination under general anesthesia:
 - 5.12.1.1. Inject acepromazine 0.75 mg/kg and butorphanol 0.2 mg/kg subcutaneously. Wait 15 to 20 minutes.
 - 5.12.1.2. Maintain anesthesia with isoflurane.
 - 5.12.1.3. Exsanguinate via cardiac puncture.
 - 5.12.2. Collect 5.6 mL/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.

SOP REVISION HISTORY

DATE	NEW VERSION
2016.09.22	5.4.1. Tranquilize rabbits by injecting intramuscularly (can be mixed do not mix acepromazine and buprenorphine in the same syringe):
2016.09.22	5.4.2. Apply Consider applying EMLA cream over the ear on the blood collection site 15-30 minutes before puncture.
2016.09.22	5.4.1.2. Butorphanol (0.2 mg/kg) or buprenorphine (0.2 0.1 mg/kg),
2022.01.13	3.7. Butorphanol or buprenorphine
2022.01.13	3.8. Dexmedetomidine
2022.01.13	3.9. Atipamezole
2022.01.13	4.1. The PI must prepare an immunogen that is must be: