

FLSC Standard Operating Procedure for Antibody Production in Rats Using TiterMax Adjuvant

Animals:

1. Male or female LOBUND –Wistar rats (or similar strain at the request of the principal investigator) between the ages of 6 – 20 weeks at the time of the initial immunization.
2. Animals will be sourced from approved vendors or from in-house breeding colonies.
3. When purchased from a commercial vendor, animals must be acclimated for at least one week.

Antigen Preparation:

1. Antigens for injection into rats are prepared by the investigator using the following guidelines:
 - a. The antigen must be filter sterilized.
 - b. The antigen must be given to the FLSC staff in vials that facilitate sterile removal of the antigen. (i.e. rubber-capped vacutainers, or eppendorf tubes).
2. Antigen preparations include the use of adjuvants such as TiterMax® to aid in the stimulation of the immune response.
3. When using TiterMax® it is recommended that only the initial injection contains adjuvant.
4. Subsequent booster immunizations may consist of the antigen in an aqueous solution or half the initial immunization volume of antigen + TiterMax®.
5. The TiterMax® is matched in volume to the antigen, making a 1:1 mixture.
 - a. This mixture must be thoroughly emulsified by passing the antigen and adjuvant through a 20 – 22 gauge needle on a 3cc syringe. Some labs use sonification for complete emulsification.
 - b. Avoid adding air.
6. All antigen preparations must be labeled with the name of the antigen, the name of the PI and the date.
7. Antigens may be stored at FLSC or the antigen can be given to FLSC staff on the day of the scheduled injection.

Injection Procedure:

1. Intramuscular (IM) injections are used for the initial injection and subsequent boosts. The maximum total volume (antigen + adjuvant) for IM injection is 100 microliters divided equally between the hind leg quadriceps.
2. Test bleeds are required prior to boosting and are recommended at 21 days to 28 days post-immunization. The test bleed will indicate whether a boost is needed or if blood collection for antibody harvest can begin.
3. When the antibody titer is high, it is not necessary that a booster be given. Harvest of blood for antibody collection can begin. When the titer is low, the booster is given IM with half or less of the immunizing dose of TiterMax®. Booster injections are distributed in both hind leg quadriceps.
4. By request of the PI and with IACUC approval, a final boost can be given intravenously (IV). Final IV boosters cannot contain adjuvant; instead a sterile saline solution containing only the antigen is administered. It is vital that any IV injection be free of debris or contaminants. The maximum volume for the IV injection is 0.4 ml.
5. The immunization schedule can vary from injections at 21day to 6 week intervals. It is the responsibility of the investigator to supply FLSC with a schedule and to submit Procedure Request Forms with the date, time and animal identification for FLSC staff to perform animal procedures.
6. The repeated injection of TiterMax® in animals with high antibody titers has been reported to cause Arthus reactions. This condition is painful and any animals exhibiting signs of pain or distress will be evaluated by the Attending Veterinarian.
7. Rats will be euthanized earlier if they meet criteria listed in the IACUC's Humane Endpoints in Animal Experimentation or if medically warranted by the Attending Veterinarian.

Bleeding Procedures:

1. Blood collection is via the orbital sinus according to FLSC SOPs.
2. Orbital sinus blood collection requires rats be anesthetized.
3. Blood samples are collected in eppendorf tubes.
4. The maximum blood volume collected at one time is 0.5 ml. The standard volume is 0.2 ml.
5. A test bleed can be performed at the request of the investigator prior to the start of immunizations. Pre-bleeds are taken for screening purposes or to establish a baseline for comparison to subsequent post-immunization blood samples.
6. Rats cannot be bled more often than once weekly unless scientifically justified in the IACUC approved animal use protocol. If blood is needed more often animals will be monitored for anemia using packed cell volumes.

Fusions:

1. FLSC staff can assist investigators by exsanguinating the rat under CO2 narcosis and performing a sterile harvest of the spleen. Prior to tissue harvest the animals must have the chest cavity opened bilaterally to ensure death.
2. It is the investigator's responsibility to provide sterile media and ice in an appropriate container for the spleen.
3. Sterile instruments for harvesting the spleen are required. These should be supplied by the investigator or arranged prior to the date of harvest with FLSC management.

Suggested Immunization Schedule

Immunization Schedule	Procedure
Day 0	Pre-bleed
Day 0	1 st Immunization antigen+adjuvant IM 100 microliter
Day 28	1 st Test Bleed
Day 30	Boost antigen+adjuvant IM 50 microliters OR Antibody Harvest (small bleed or exsanguination)
Day 58	2 nd Test Bleed
Day 60	Boost antigen+adjuvant IM 50 microliters OR Antibody Harvest (small bleed or exsanguination)

Immunization dose, routes, schedule as recommended by TiterMax USA, Inc.

4/8/1997 VAS, rev. 11/1998 MAS, 8/2004, 3/2007, 10/2016 VAS
APR Antibody TiterMax SOP16