Standard Operating Procedure for Antibody Production in Rats Using TiterMax Adjuvant

Type Used: Male or female LOBUND –Wistar rats (or other strains at the request of the principal investigator) between the ages of 6 – 20 weeks at the time of the initial immunization.

Purchased From: Born in house or purchased from an approved vendor.

Availability: When purchased from a commercial vendor, must be acclimated for at least one week.

Antigen Preparation:

1. Antigens used for injection into rats are prepared by the individual labs according to these guidelines:
   a. The antigen must be filter sterilized.
   b. The antigen must be given to FLSC staff in vials or containers that facilitate sterile removal of the antigen. (i.e. rubber-capped vacutainers, or eppindorf tubes).
2. Antigen preparation includes the use of adjuvants to aid in the stimulation of the immune response.
   a. When using TiterMax it is recommended that only the initial injection contains adjuvant.
   b. Subsequent booster immunizations may consist of the antigen in an aqueous solution or half the initial immunization volume of antigen + TiterMax.
   c. The TiterMax is matched in volume to the antigen, making a 1:1 mixture. The antigen and adjuvant must be emulsified thoroughly. Some labs use sonification for complete emulsification.
3. All antigen preparations must be labeled with the name of the antigen, the name of the PI and the date.
4. Antigens may be stored at FLSC in the refrigerator or brought to FLSC 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. This test bleed will indicate whether a boost is needed or if blood collection for antibody harvest can begin.
3. If the antibody titer is high, it is not necessary to boost and harvest of antibody can begin. If the titer is low, you may boost IM with half or less of the immunizing dose of **TiterMax®** distribute the injections among several sites.
4. By request of the PI and with IACUC approval, a final boost can be given intravenously (IV). The IV boost cannot contain adjuvant. It is usually a sterile saline solution containing only the antigen. It is vital that any IV injection be free of debris or contaminants. Maximum IV injection volume is 0.4 ml.
5. The immunization schedule can vary from injections at 21day to 6 week intervals. Generally a schedule of 28 day intervals between injections is used. It is the responsibility of the PI to supply FLSC with an injection schedule and to fill out Procedure Request Forms with the date, time and animal identification if FLSC is to perform animal procedures.
6. Injections are given according to the SOP in the FLSC procedure guide. Briefly, the rat is restrained manually with fingers on each side of the head and the remaining fingers and thumb circling the thorax. The injection is given in the quadriceps muscle in both hind legs. The injection should be made slowly and smoothly being sure not to insert the needle too deeply.

The maximum volume for IM antigen + adjuvant injections is less than the maximum for other types of injections. Note: The repeated injection of TiterMax in rats 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. Euthanasia may occur earlier based upon the IACUC's Humane Endpoints in Animal Experimentation guidelines if untoward reactions occur.

Bleeding Procedures:

1. All pre-bleeds and test bleeds are taken via the orbital sinus in accordance with the SOP in the FLSC procedure guide. Briefly, the rats are anesthetized with an inhalant anesthetic. The anesthetized rat has a hematocrit tube inserted in the medial canthus of the eye and directed caudally behind the globe to the medial-posterior aspect of the orbit. The tube is firmly rotated to cut the conjunctival membranes and the vascular plexus. Blood will flow into the hematocrit tube. For collections of less than 0.5 ml, 3 to 5 hematocrit tubes are filled, sealed and spun. For maximum blood volume collections of 0.5 ml, the blood is allowed to flow through the hematocrit tube and into a microtainer tube. The microtainer tubes are capped and spun for separation of the blood into liquid and cellular portions.
2. A test bleed can be performed at the request of the PI prior to the start of the immunizations. Pre-bleeds are taken for screening purposes or to establish a baseline for comparison to subsequent post-immunization blood samples.
3. Rats cannot be bled more often than once weekly unless scientifically justified in the IACUC approved animal use protocol. If rats are bled more often, PCV's will be monitored closely for anemia. The maximum blood volumes for collection per adult rat are:

Once every 3-7 days (1% BV)	Once every 14 days (5% BV)	Once a month (10% BV)
0.20 ml	0.9 ml	1.8 ml
3 x 75Φl hematocrit tube	12 x 75Φl hematocrit tube 1 microtainer tube	2 microtainer tubes

Fusions:

1. Fusions are usually scheduled 3-5 days after the IV booster injection.
2. Rats will be euthanized with CO_2 just prior to bleeding and organ harvest.
3. FLSC staff will exsanguinate and harvest the spleen on the scheduled day at the scheduled time.
4. It is the PI's responsibility to provide culture media and ice in appropriate containers for storage of the spleen.
5. FLSC will provide sterile instruments for harvesting the spleen.

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