

## **FLSC Standard Operating Procedure for Antibody Production in Mice Using TiterMax Adjuvant**

### **Animals:**

1. Male or female Balb/C (or similar strain at request of the principal investigator) should be used 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 mice 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 vacutainer tubes 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 for pending use may be stored at FLSC or the antigen can be given to FLSC staff on the day of injection.

### **Injection Procedures:**

1. Initial injections are given subcutaneous (SC) at the base of the tail with a maximum volume of 40 microliters. Alternatively, intramuscular (IM) injections of a maximum of 40 microliters divided equally between the hind leg quadriceps for an injection volume of 20 microliters into each hind leg can be used.
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 recommended that the booster be given SC with an aqueous solution of antigen without adjuvant. When the titer is low, the booster is given SC with half or less of the immunizing dose of TiterMax®. In both cases, distribute the injections among several sites along the dorsal back.
4. By request of the PI and with IACUC approval, a final booster 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 0.2 ml.
5. Immunization schedules vary from 21 days 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 the 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. Mice 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 or facial vein according to FLSC SOPs.
2. Orbital sinus blood collection requires mice be anesthetized.
3. With facial vein or submandibular blood collection mice are manually restrained. No anesthesia is required.
4. Blood samples are collected in microhematocrit tubes.
5. The maximum blood volume collected at one time is three (3) microhematocrit tubes (75µl total volume/tube). The standard collection volume is two microhematocrit tubes.
6. Mice cannot be bled more than once weekly unless scientifically justified in the approved IACUC 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 mice under CO<sub>2</sub> narcosis and performing a sterile harvest of the spleen. Prior to tissue harvest the animals must be cervically dislocated 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 <sup>st</sup> Immunization antigen+adjuvant IM or SQ 40 microliter
Day 28	1 <sup>st</sup> Test Bleed
Day 30	Boost antigen only SQ 40 microliters or antigen+adjuvant IM 20 microliters OR Antibody Harvest (small bleed or exsanguination)
Day 58	2 <sup>nd</sup> Test Bleed
Day 60	Boost antigen only SQ 40 microliters or antigen+adjuvant IM 20 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  
AP.M Antibody TiterMax SOP16