

Standard Operating Procedure for Antibody Production in Mice Using TiterMax Adjuvant

Type Used: Male or female Balb/C (or similar strain at request of principal investigator) between the ages of 6 – 20 weeks at the time of the initial immunization.

Purchased From: Harlan Sprague Dawley (HSD) or other approved source.

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

Antigen Preparation:

1. Antigen used to inject into the mice is prepared by the individual labs that are using the mice. The following guidelines are to be used:
 - 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 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 Procedures:

1. In general, a single subcutaneous (SQ) injection at the base of the tail with a maximum volume of 40 microliters is given for the first injection. 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 for the first immunization.
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 recommended that the boost be done SQ with an aqueous solution of antigen without adjuvant. If the titer is low, you may boost SQ with half or less of the immunizing dose of **TiterMax®**. In both cases, distribute 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 0.2 ml.
5. Immunization schedules vary from 21 days to 6 week intervals. It is the responsibility of each lab to supply FLSC with its schedule and to fill out Procedure Request Forms with the date, time and animal identification if FLSC is to perform the animal procedures.
6. Injections are given according to the FLSC SOP (Procedure for Subcutaneous Injection in the Mouse and Procedure for Intramuscular Injection in the Mouse). Briefly, the mouse is restrained

manually by scruffing the fur at the neck and shoulders. The injection is given SQ at the base of the tail. The injection should be made slowly and smoothly being sure not to insert the needle too deeply. If the intramuscular (IM) route is used, the mouse will be anesthetized according to the FLSC Guidelines for Anesthesia and Analgesia using inhaled Isoflurane. 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 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. Euthanasia may occur earlier based upon the IACUC's Humane Endpoints in Animal Experimentation guidelines if untoward reactions occur.

Bleeding Procedures:

1. Bleeding is done via the orbital sinus or facial vein (Procedure for Submandibular Blood Sampling and Blood Withdrawal Using the Orbital Sinus). Briefly for retroorbital bleeds, mice are anesthetized and a hematocrit tube is inserted at the medial canthus of the eye and directed caudally behind the globe to the medial- posterior aspect of the orbit. The tube is lightly twisted to disrupt the vascular plexus at this site and blood collected as it flows out with the hematocrit tube. For facial vein or submandibular bleeds, the mouse is manually restrained and a special lancet is used to puncture the facial vein located on the lower cheek behind the curve of the mandible. The blood is collected in a microhematocrit tubes as it flows out. The maximum volume collected at one time is three (3) microhematocrit tubes (75µl total volume/tube); two tubes is the standard amount needed.
2. Mice cannot be bled more than once weekly, unless scientifically justified in the IACUC approved animal use protocol. If blood is needed more often, PCV's will be monitored closely.

Fusions:

1. On the scheduled day of fusion, FLSC staff will exsanguinate the mouse and harvest the spleen. The mice are euthanized with CO₂ prior to the bleeding.
2. It is the principal investigator's responsibility to provide media and ice in an appropriate container for the spleen.
3. FLSC will provide sterile instruments for harvesting the spleen.

Suggested Immunization Schedule

Immunization Schedule	Procedure
Day 0	Pre-bleed
Day 0	1 st Immunization antigen+adjuvant IM or SQ 40 microliter
Day 28	1 st 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 nd Test Bleed
Day 60	Boost antigen only SQ 40 microliters antigen+adjuvant IM 20 microliters OR Antibody Harvest (small bleed or exsanguination)

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

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