

## **Standard Operating Procedure for Antibody Production in Mice Using Freund's 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 vacutainer tubes or eppendorf tubes).
2. Antigen preparations include the use of adjuvants - Complete Freund's Adjuvant (CFA) and Incomplete Freund's Adjuvant (ICFA) to aid in the stimulation of the immune response. Complete Freund's Adjuvant is used in the first injection only. Subsequent booster immunizations use ICFA. The CFA and ICFA are matched in volume to the antigen, making a 1:1 mix. This mix must be thoroughly emulsified.
3. All antigen preparations must be labeled with the name of the antigen, name of the PI and the date.
4. Antigens can be stored at FLSC in the refrigerator or each volume of antigen can be given to the FLSC staff on the day of injection.

### **Injection Procedures:**

1. In general, intraperitoneal (IP) injections with a maximum volume of 0.25 ml (antigen + CFA) are given for the first injection. The subsequent IP boosts (antigen+ ICFA) are given at 14 day intervals.
2. 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. The maximum volume for the IV injection is 0.2 ml.
2. Injection schedules vary from 10 days to 2-3 week intervals. It is the responsibility of the PI to supply FLSC with an schedule and to fill out Procedure Request Forms with the date, time and animal identification if FLSC is to perform animal procedures.
3. IP injections are given according to the IP injection SOP in the FLSC procedure guide. Briefly, the mouse is grasped and tilted, head-downward, at a 45° angle. The injection is given in the abdomen (alternating sides for booster immunizations) and the needle inserted perpendicular to the spine in the area bordered by the midline, groin and the top of the hip (these land marks delineate a triangular injection area). Before injection, the syringe should be aspirated to assure that the needle is not within a blood vessel or a loop of bowel. The injection should be made smoothly and slowly. The maximum volume for an IP injection is 0.25cc.
4. Complete Freund's Adjuvant (CFA) causes peritonitis. Thus the antigen injection schedule should be minimized to the shortest possible time frame. The mice should be humanely euthanized within 12 weeks of the injection of CFA.
5. Mice will be euthanized earlier if they meet the criteria in the IACUC's Humane Endpoints in Animal Experimentation guidelines if untoward reactions occur.

### **Bleeding Procedures:**

1. Test bleeds are done by FLSC staff 10 days after the second boost, and then as scheduled

- by the principal investigator.
2. 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 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.
  3. 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 CO<sub>2</sub> 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 <sup>st</sup> Immunization antigen + CFA IP
Day 14	1 <sup>st</sup> Boost antigen + ICFA IP
Day 28	2 <sup>nd</sup> Boost antigen + ICFA IP <b>or</b> Exsanguination
Day 38	1 <sup>st</sup> Test Bleed
Day 42	3 <sup>rd</sup> Boost antigen + ICFA IP <b>or</b> Exsanguination
Day 52	2 <sup>nd</sup> Test Bleed or Exsanguination