

FLSC Standard Operating Procedure for Antibody Production in Mice Using Freund's 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 - Complete Freund's Adjuvant (CFA) and Incomplete Freund's Adjuvant (ICFA) to aid in the stimulation of the immune response.
3. Complete Freund's Adjuvant (CFA) is used for the first injection only.
4. Subsequent booster immunizations use Incomplete Freund's Adjuvant (ICFA).
5. The CFA and ICFA are 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 3 cc syringe.
 - b. Avoid adding air.
 - c. The mixture will become opaque white when well mixed.
6. All antigen preparations must be labeled with the name of the antigen, 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 intraperitoneal (IP) with a maximum volume of 0.25 ml (antigen +CFA). Subsequent IP boosters (antigen+ ICFA) are given at 14 day intervals.
2. 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.
3. Injection schedules vary from 10 days to 2-3 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.
4. IP injections are given in the abdomen. The injections are given on alternating sides for booster immunizations. The maximum volume for an IP injection is 0.25ml.
5. Complete Freund's Adjuvant (CFA) causes peritonitis. The injection schedule should be compressed to the shortest time frame possible. Mice should be humanely euthanized within 12 weeks of the initial antigen injection.
6. 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. Test bleeds are done 10 days after the second antigen booster, and then as scheduled by the investigator.
2. Blood collection is via the orbital sinus or facial vein according to FLSC SOPs.
3. Orbital sinus blood collection requires mice be anesthetized.
4. With facial vein or submandibular blood collection mice are manually restrained. No anesthesia is required.
5. Blood samples are collected in microhematocrit tubes.

6. 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.
7. 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 CO₂ 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 st Immunization antigen + CFA IP
Day 14	1 st Boost antigen + ICFA IP
Day 28	2 nd Boost antigen + ICFA IP or Exsanguination
Day 38	1 st Test Bleed
Day 42	3 rd Boost antigen + ICFA IP or Exsanguination
Day 52	2 nd Test Bleed or Exsanguination