

FLSC Standard Operating Procedure for Antibody Production in Rats Using Freund's Adjuvant

Animals:

1. Male or female LOBUND –Wistar rats (or similar strain at the 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 rats 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 vacutainers, or eppendorf tubes).
2. Antigen preparation includes 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. All subsequent booster immunizations use Incomplete Freund's Adjuvant (ICFA).
5. The CFA or 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, 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 Procedure:

1. Initial injections are given intraperitoneal (IP) with a maximum volume of 0.5 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 0.4 ml for adult rats.
3. Injection schedules can vary from 10 days to 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 0.5 ml.
5. Complete Freund's Adjuvant (CFA) in the rat has been reported to cause disseminated granulomas in lung, liver, heart, kidney, lymph nodes and skeletal muscle as well as peritonitis and adhesions. These conditions tend to worsen with time. Rats should be humanely euthanized within 12 weeks of the initial antigen injection.
6. Rats 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 according to FLSC SOPs.
3. Orbital sinus blood collection requires rats be anesthetized.
4. Blood samples are collected in eppendorf tubes.
5. The maximum blood volume collected at one time is 0.5 ml. The standard volume is 0.2 ml.

6. A test bleed can be performed at the request of the investigator prior to the start of immunizations. Pre-bleeds are taken for screening purposes or to establish a baseline for comparison to subsequent post-immunization blood samples.
7. Rats cannot be bled more often 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 rat under CO2 narcosis and performing a sterile harvest of the spleen. Prior to tissue harvest the animals must have the chest cavity opened bilaterally 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