

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

Type Used: Male or female LOBUND –Wistar rats (or other strains at the request of the principal investigator) between the ages of 6 – 20 weeks at the time of the initial immunization.

Purchased From: Born in house or purchased from an approved vendor.

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

Antigen Preparation:

1. Antigens used for injection into rats are prepared by the individual labs using the rats according to these guidelines:
 - a. The antigen must be filter sterilized.
 - b. The antigen must be given to FLSC staff in vials or containers that facilitates sterile removal of the antigen (i.e. rubber-capped vacutainers, or eppendorf tubes).
2. Antigen preparation includes the use of adjuvants to aid in the stimulation of the immune response.
 - a. When using Complete Freund's Adjuvant (CFA) only the initial injection contains CFA.
 - b. All subsequent booster immunizations must use Incomplete Freund's Adjuvant (ICFA).
 - c. The CFA or ICFA are 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 Procedure:

1. In general, intraperitoneal (IP) injections are used for the initial injection and all subsequent boosts. The maximum total volume for IP injection is 0.5 ml.
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. Maximum IV injection volume is 0.4 ml for adult rats.
3. The immunization schedule can vary from injections at 10 day to 3 week intervals. Generally a schedule of 14 day intervals between injections is used. It is the responsibility of the PI to supply FLSC with an injection schedule and to fill out Procedure Request Forms with the date, time and animal identification if FLSC is to perform animal procedures.
4. Injections are given according to the FLSC SOP (Procedure for Intraperitoneal Injection of the Rat). Briefly, the rat is restrained manually with the head tilted downward at a 45E angle. The injection is given in the lower quadrant of the abdomen on either side. The needle is inserted perpendicular to the plane of the spine in the area bordered by the midline, groin and top of the hip (these landmarks delineate a triangular injection area). The injection should be made slowly and smoothly being sure not to insert the needle too deeply. Note: Use of 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.
5. Rats should be euthanized within 12 weeks of the injection of CFA. Euthanasia may occur earlier based upon 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. All pre-bleeds and test bleeds are taken via the orbital sinus in accordance with the FLSC SOP (Blood Withdrawal Using the Orbital Sinus). Briefly, the rats are anesthetized with an inhalant anesthetic. The anesthetized rat has a hematocrit tube inserted in the medial canthus of the eye and directed caudally behind the globe to the medial-posterior aspect of the orbit. The tube is firmly rotated to cut the conjunctival membranes and the vascular plexus. Blood will flow into the hematocrit tube. For collections of less than 0.5 ml, 3 to 5 hematocrit tubes are filled, sealed and spun. For maximum blood volume collections of 0.5 ml, the blood is allowed to flow through the hematocrit tube and into a microtainer tube. The microtainer tubes are capped and spun for separation of the blood into liquid and cellular portions.
3. A test bleed can be performed at the request of the PI prior to the start of the immunizations. Pre-bleeds are taken for screening purposes or to establish a baseline for comparison to subsequent post-immunization blood samples.
4. Rats cannot be bled more often than once weekly unless scientifically justified in the IACUC approved animal use protocol. If rats are bled more often, PCV's will be monitored closely for anemia. The maximum blood volumes for collection per adult rat are:

Once every 3-7 days (1% BV)	Once every 14 days (5% BV)	Once a month (10% BV)
0.20 ml	0.9 ml	1.8 ml
3 x 75Φl hematocrit tube	12 x 75Φl hematocrit tube 1 microtainer tube	2 microtainer tubes

Fusions:

1. Fusions are usually scheduled 3-5 days after the IV booster injection.
2. Rats will be euthanized with CO₂ just prior to bleeding and organ harvest.
3. FLSC staff will exsanguinate and harvest the spleen on the scheduled day at the scheduled time.
4. It is the PI's responsibility to provide culture media and ice in appropriate containers for storage of the spleen.
5. 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 + 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