

**Stony Brook University  
Institutional Animal Care and Use Committee (IACUC)**

**HYBRIDOMA PRODUCTION**

**A. General Information**

The IACUC has approved the following protocol for mouse hybridoma production to be performed by the Cell Culture/Hybridoma Facility at Stony Brook University.

There are no methods readily available for the procedure of producing hybridoma cell lines without using animals for the source of spleen cells. The initial fusion is performed with parent mouse myeloma cells which do not secrete antibodies and mouse pre-immune spleen cells. The goal is to generate monoclonal antibodies that recognize the antigen of choice.

See [www.nal.usda.gov/awic/pubs/antibody/overview.htm](http://www.nal.usda.gov/awic/pubs/antibody/overview.htm) for more information on adjuvants.

It is also possible to produce high concentration monoclonal antibodies without the use of animals. Please see <http://www.stonybrook.edu/cchf> for more information. This service can also be provided by the Cell Culture/Hybridoma Facility at Stony Brook University.

**B. Procedures**

- 1) Balb/c mice are used for the source of antigen-specific spleen cells. Animal housing and technical procedures are provided by the DLAR. The mice are injected IP with amounts of antigen between 10 -100 ug per mouse in desired adjuvant. Adjuvants are used to insure the successful immunization of the animals with the antigen. The following adjuvant regimens may be used for immunization:
  - a) **RIBI adjuvant** - (200ul IP; every two weeks; four injections total)
  - b) **Freunds Complete adjuvant** - (100ul IP; one injection only; followed by Freunds Incomplete adjuvant - (100ul IP, every two weeks; two injections)
  - c) **Titermax adjuvant**- (100ulIP; every three weeks; two injections)
- 2) One week following the last injection, each mouse is anesthetized with Isoflurane (5%) and bled retro-orbitally, following the methodology and guidelines listed in the Retro-orbital Rodent Blood Sampling Standard Procedure.
- 3) The mouse serum is tested for an antibody titer. If the response is not at the level needed for a successful fusion (1:1000 dilution) the animal is inoculated again IP. If a mouse shows a high titer, the animal is rested for 3-4 weeks allowing time for the titer to drop. A final antigen boost is then given without adjuvant.

- 4) The mouse is euthanized four days post-boost, according to the standard procedure. The spleen is removed and the fusion performed. The additional mice that were injected with the antigen of interest will be kept for approximately six months until the success of the fusion can be fully determined.

### **C. Anesthesia**

- 1) Anesthesia is required for all retro-orbital bleeds.
- 2) Isoflurane is the preferred agent (5%, by drop method or 2-3% by precision vaporizer).
- 3) Ketamine (40-120 mg/kg) and Xylazine (10mgf/kg) IP may also be used.
- 4) The use of any other anesthetic agents must be listed in the IACUC application.

### **D. Anesthetic Monitoring**

During blood collection, the following parameters must be continually monitored:

- Respiratory rate
- Spontaneous movement
- Response to noxious stimulus (ie. Toe-pinch)

### **E. Anesthesia Recovery Monitoring**

- 1) During recovery from anesthesia, the following parameters must be monitored at a minimum of 15 minute intervals until the animal is ambulatory:
  - Respiratory rate
  - Movement
  - Ability to maintain sternal recumbancy
- 2) Animals should be placed on a water re-circulating heating blanket, or well covered, to conserve body temperature. To protect the animals from hypothermia they should never be placed on metal surfaces
- 3) Animal should recover in 10-15 minutes if gas anesthesia is used and within 1-2 hours if injectable anesthetics are used.

### **F. Analgesia**

None required, topical analgesia may be used following retro-orbital blood sampling.

### **G. Potential Adverse Effects**

- 1) Adjuvants can cause localized inflammation and swelling in the abdominal cavity. Mice should be monitored for: weight loss, abdominal pain, respiratory distress (labored breathing or increased respiratory rate) and abdominal distension.
- 2) Retroorbital bleeding can cause eye infection, peri-orbital swelling, redness, hematoma formation and blindness.

## **H. Clinical Monitoring and Management**

- 1) Animals should be monitored at least twice weekly after adjuvant injections and after each retro-orbital bleed.
- 2) If adverse effects are seen, the investigator should consult immediately with the DLAR veterinary staff regarding treatment options. If animals have acute adverse reactions to the anesthetic agents (respiratory distress and/or lack of recovery), they must be euthanized immediately.
- 3) Animals visually appearing to lose weight should be weighed every other day.

## **I. Early Endpoints**

Animals will be immediately euthanized if:

- a) the eyeball is acutely damaged, if treatment of an injured/infected eye is unsuccessful, and/or if bilateral blindness occurs.
- b) weight loss greater than 15% baseline occurs.
- c) the animal is experiencing respiratory distress or abdominal distension.