

## ACORP Appendix 2, DRAFT ANTIBODY PRODUCTION

1. **Monoclonal Antibody Production.** Will monoclonal antibodies be produced in animals or harvested from hybridoma cell lines as part of this project?

- No. Proceed to item 3.  
 Yes. Complete item 1.a.

a. Is antibody harvest limited to existing hybridoma cell lines with no further immunizations or lymphocyte fusions planned?

- Yes. Proceed to item 2 below.  
 No. Fill out items 1.b and 1.c below; then proceed to item 2.

b. Complete the following table regarding the immunization protocol for the animals prior to lymphocyte harvest for hybridoma creation. For each antigen for which multiple immunization days will be used, use a separate row in the table for each immunization day.

Injection day (e.g. day 0, 7, 30, etc.)	Antigen	Total amount (mg) and volume (ml) of antigen injected	Identity and volume (ml) of adjuvant injected	Total injection volume per animal (antigen plus adjuvant; ml)	Divided into how many injections?	Injection route and location of injections on body

c. If feeder cells for supporting hybridoma colony growth will be collected from animals, describe the exact procedures that will be used to collect the feeder cells and the number of animals needed for this purpose.

2. You must consider alternate research methods that can replace the use of animals. Will any animals be used to expand hybridoma cell lines so that antibody can be harvested from ascites fluid?

- No. Proceed to item 2.d.  
 Yes. Complete items 2.a-2.c below; then proceed to item 2.d.

a. Explain why *in vitro* cell culture systems for harvesting monoclonal antibodies are not adequate to meet the research objectives.

b. Complete the following table.

Hybridoma cell line designation	Number of animals used for ascites production	Priming agent and volume	Number and timing of priming injections	Volume of injected hybridoma cells	Number of abdominal taps before euthanasia

c. What criteria will be used to determine if animals should be euthanatized prior to the last

planned abdominal tap?

- d. **Blood collection.** Will survival blood collections be obtained from animals following immunization or as a “pre-bleed” prior to immunization?
- No. Proceed to item 3.
- Yes. Complete items 1) and 2) below; then proceed to item 3.

1) Complete the following table; include any “pre-bleeds” prior to immunizations.

Site of blood collection	Amount of blood collected expressed as volume (ml) and % of body weight (assume 1 ml weighs 1 gram)	Number of blood collections	Interval between collections

- 2) Will anesthetics, tranquilizers, or analgesics be used prior to blood collection?
- No. Justify the omission of pain-relieving agents below; then proceed to item 3.
- Yes. Describe the administration of pain-relieving agents including dose (mg/kg), volume (ml), route, and frequency/duration; then proceed to item 3.

3. **Polyclonal Antibody Production.** Will polyclonal antibodies be produced in this species of animal as a part of this project?
- No. **Do not** complete items 3.a.-3.c. Go to item 4 below.
- Yes. Complete items 3.a.-3.c., then go to item 4.

a. Complete the following table. For each antigen for which multiple immunization days will be used, use a separate row in the table for each day.

Injection day (e.g. day 0, 7, 30, etc.)	Antigen	Total amount (mg) and volume (ml) of antigen injected	Identity, concentration and volume (ml) of adjuvant injected	Total injection volume per animal (antigen plus adjuvant; ml)	Divided into how many injections?	Injection route, and location of injections on body

- b. List possible adverse effects in animals that might be seen from the proposed antigen or adjuvant injections and what measures will be taken should these adverse effects occur.
- c. **Blood collection.** Will survival blood collections be obtained from animals following immunization or as a “pre-bleed” prior to immunization?
- No. Proceed to item 4.
- Yes. Complete items 3.c.1) and 2); then proceed to item 4.

1) Complete the following table; include any “pre-bleeds” prior to immunizations.

Site of blood collection	Amount of blood collected expressed as volume (ml) and % of body weight (assume 1 ml weighs 1 gram)	Number of blood collections	Interval between collections

2) Will anesthetics, tranquilizers, or analgesics be used prior to blood collection?

- No. Justify the omission of pain-relieving agents below; then proceed to item 4.  
 Yes. Describe the administration of pain-relieving agents including dose (mg/kg), volume (ml), route, and frequency/duration below; then proceed to item 4.

4. **Terminal blood collection.** Will animals used for antibody production be exsanguinated as a method of euthanasia?

- No. Go to item 5.  
 Yes. Complete items 4.a, b., and c. below, then go to item 5.

a. Describe the method of exsanguination.

b. Will anesthetics, tranquilizers, or analgesics be used prior to exsanguination?

- No. Justify the omission of pain-relieving agents below; then proceed to item 5.  
 Yes. Describe the administration of pain-relieving agents including dose (mg/kg), volume (ml), route, and frequency/duration here; then proceed to item 5.

c. How will you make sure that the animals are dead following blood withdrawal?

5. How will the antigens or cell lines listed in items 1.b., 2.b., and 3.a. be screened to make sure they do not harbor infectious agents that could infect other laboratory animals or people after injection?

6. Return to the main ACORP form and continue with item P.