**ACORP Appendix 2**

**Antibody Production**

**Version 4**

See ACORP App. 2 Instructions, for more detailed explanations of the information requested.

1. **Immunization.** Provide the information requested below for any animals to be used for raising antibodies specifically for use in this protocol.
	1. Describe the immunization protocol in the table below, using a separate row for each day on which any agent (including primer, antigen, and/or adjuvant) will be administered. (Make sure that each primer, antigen, and adjuvant is also included in Appendix 3.)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Immun-ization day (e.g. day -7, 0, 7, 30, etc.) | Antigen | Adjuvant – give name, concentration, and volume (ml)  | Total injection volume (ml) per animal (antigen plus adjuvant) | Divided among how many injection sites? | Injection route and location of injection site(s) on body |
| Name | Total amount (mg) and volume (ml) |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |

* 1. Describe how each antigen will be screened to make sure that it does not harbor infectious agents that could infect other laboratory animals or people after injection.

►

* 1. List possible adverse effects that might be observed in animals receiving the proposed primer, antigen, and/or adjuvant injections, and describe the measures that will be taken if these adverse effects occur:

►

* 1. Give the justification for using any primer or adjuvant that is expected to cause pain or distress in the animals.

►

1. **Survival Blood Collection.** Will blood be collected as a survival procedure for the production and harvesting of antibodies on this protocol?

► ( ) No, the production and harvest of antibodies on this protocol does not involve survival collection of blood.

► ( ) Yes, this protocol requires the collection of blood in a survival procedure, before (as a “pre-bleed”) and/or after immunization. Make sure this is included in Item R of the ACORP, and complete items 2.a, 2.b, and 2.c, below.

* 1. Describe each survival collection of blood in the table below, including any “pre-bleeds” prior to immunizations:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Site of Blood Collection | Amount of Blood Collected at any one time,expressed as volume (ml) and as % of body weight (assume 1 ml = 1 gram) | Number of Blood Collections | Time Interval(s) Between Successive Collections | Volume Replace-ment?(yes/no) |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |

* 1. Will anesthetics, tranquilizers, or analgesics be administered for blood collection?

► ( ) No anesthetics, tranquilizers, or analgesics will be administered for blood collection. Explain why it is appropriate or necessary NOT to administer pain-relieving agents:

►

► ( ) Yes. Describe the administration of pain-relieving agents, including the name of each agent, and its dose (mg/kg), volume (ml), and route and frequency/duration of administration (Make sure this information is also included in Appendix 3):

►

* 1. Will volume replacement be provided for blood that is collected?

► ( ) Volume will NOT be replaced for some of the blood collection listed. For each collection listed in Item 2.a, above, for which volume will NOT be replaced, explain why not.

►

► ( ) Volume WILL be replaced for some of the blood collection listed. For each collection listed in Item 2.a, above, for which volume WILL be replaced, describe the replacement(s) that will be provided (including the composition of the replacement(s), volume, and route of administration).

►

1. **Terminal Blood Collection.** Will animals be euthanatized by exsanguination, for harvest of antibodies?

► ( ) No, this protocol does NOT involve terminal blood collection for harvest of antibodies.

► ( ) Yes, this protocol DOES require terminal blood collection for the harvest of antibodies. Make sure this is included in Item R of the ACORP, and complete Items 3.a., 3. b., and 3.c., below:

* 1. Describe the method(s) to be used for euthanasia and exsanguination:

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* 1. Will anesthetics, tranquilizers, or analgesics be administered for exsanguination?

► ( ) No anesthetics, tranquilizers, or analgesics will be administered for the exsanguination(s). Explain why it is appropriate or necessary NOT to administer pain-relieving agents:

►

► ( ) Yes. Describe the administration of pain-relieving agents including the name of each agent, and its dose (mg/kg), volume (ml), and route and frequency/duration of administration (Make sure this information is also included in Appendix 3):

►

* 1. Describe how you will make sure that the animals are dead after collection of the blood:

►

1. **Harvesting Feeder Cells.** Describe the exact procedures (including administration of pain-relieving agents) that will be used on any donor animals from which feeder cells will be collected for this protocol, and estimate the number of animals needed for this purpose. Make sure that these animals are included in Item I of the ACORP, and that the harvesting of feeder cells is included in Item R of the ACORP.

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1. **Expansion of Hybridoma Cell Line(s) *in vivo***. Will any animals be used to expand hybridoma cell lines so that antibody can be harvested from ascites fluid?

► ( ) No animals will be used on this protocol for *in vivo* expansion of hybridoma cell lines.

► ( ) Yes, this protocol requires use of some animals for *in vivo* expansion of hybridoma cell lines. Make sure that the animals used for this are included in Item I of the ACORP, the priming agent and the hybridoma cells are documented in Appendix 3, and the collection of ascites fluid is included in Item R of the ACORP. Complete items 5.a, 5.b, and 5.c, below.

* 1. Explain why alternate research methods that do not require the use of additional animals (e.g., *in vitro* cell culture systems for harvesting monoclonal antibodies) are not adequate to meet the research objectives of this project.

►

* 1. Complete the following table to summarize the procedures to be performed in expanding the hybridoma cell lines and collecting ascites fluid:

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Hybridoma cell line designation | Number of animals to be 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 |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |

* 1. Describe the exact procedures (including administration of pain-relieving agents) that will be used for the abdominal taps to be performed on this protocol

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* 1. List the criteria for euthanasia of animals prior to the last planned abdominal tap.

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(Use Appendix 9 to document any “departures” from the standards in the *Guide* represented by these procedures. Consult the IACUC or the Attending Veterinarian for help in determining whether any “departures” are involved.)