

Antibody Production
IACUC Standard Procedure
Effective Date: June 2023



A. Monoclonal Antibody Production

This Standard Procedure does not cover in vivo production of monoclonal antibodies via IP hybridoma cell ascites production. If this method of in vivo monoclonal antibody production is required, it must be described and justified in the approved IACUC protocol. Institutional Animal Care Use Committees are expected to critically evaluate protocols which propose using the mouse ascites method, and only allow in vivo production on the basis of strong scientific justification.

IACUC Alternatives to Ascites Production of Monoclonal Antibodies:

Both the OLAW and the USDA encourage the use of alternative methods to produce monoclonal antibodies in vitro without compromising the aims of the study. UCSF has responded to this by setting up a core laboratory within the Cell Culture Facility to produce in vitro antibodies for UCSF investigators. For specific information on how to obtain in vitro antibodies, please contact the [UCSF Monoclonal Antibody Core](mailto:Michael.Lee@ucsf.edu) at (415) 502-2335 or Michael.Lee@ucsf.edu.

B. Polyclonal Antibody Production

The IACUC protocol must describe these aspects of the antibody production procedure:

1. **Antigen:** The immunizing antigen needs to present no risk of pathogenicity or toxicity to the host animal. If toxic or pathogenic antigens are necessary, their use must be justified.
2. **Adjuvant:** Adjuvants may enhance a response, however before using Complete Freund's Adjuvant (CFA), other adjuvants should be considered (Cyt, RIBI, incomplete FA, Alum). Follow the manufactures' guidelines for adjuvant use. Use of CFA may cause inflammation or necrosis in lab animals so justification should be supplied. Undesirable side effects can be reduced by using a recommended route of administration (see #3. below), small inoculum amounts, and separating injection sites. CFA should be used only for first (priming) dose.
3. **Immunization:** Injections should be subcutaneous or, in rodents, intraperitoneal. Other routes, such as foot pads, should be justified by the investigator. For multiple sites, not more than 0.05 ml per site for mice. Sites should be well separated to prevent consolidation of inflammatory responses.
4. **Blood Collection:** Maximum withdrawal should not exceed the volumes defined in the UCSF Guidelines for blood collection in [mice](#); [rats](#).
5. **Titer Sampling Routes:** For mice, use [submandibular vein](#); for other species, use a peripheral vessel. Use anesthesia if it's required per the IACUC Standard Procedure for the selected blood collection method.
6. **Vasodilation:** Radiant heat or warm water is recommended.
7. **Monitoring:** Investigators assume responsibility for ensuring their animals' health through adequate monitoring. If complications arise, you must consult veterinary staff, or alternatively euthanize animals.

Agents: This procedure may require anesthesia. All agents administered to animals should be listed in the "Agents" section of RIO.

Potential adverse effects to be considered: shock, peritonitis, inappetence, swelling, abscesses or ulceration at the injection sites

References

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Leenaars, M., & Hendriksen, C.F. (2005). Critical steps in the production of polyclonal and monoclonal antibodies: Evaluation and recommendations. *Institute for Laboratory Animal Research Journal*, 46(3), 269-79.

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