



Johns Hopkins University

Animal Care and Use Committee

Monoclonal antibody production: Ascites method¹

PURPOSE: This guideline covers ACUC approved procedures for generating monoclonal antibodies using the ascites method in mice.

METHOD:

1. In vitro methods of monoclonal antibody production should be considered the default.² Veterinarians from [Research Animal Resources](#) are available for consultation to discuss options for monoclonal antibody production.
2. If in vitro methods fail and a decision is made to produce ascites the following should be met:
 - a. Individuals performing the procedure must be trained and skilled.
 - b. Individuals must be familiar with recognition of pain and distress in mice and should seek advice from an institutional veterinarian.
 - c. Mice should be weighed at inoculation, and at least daily from day 5 post-inoculation.
 - d. Healthy adult mice used in the study must not gain more than 25% of their body weight.
 - e. Mice that are slow to gain weight or have hemorrhagic/cloudy ascites should be monitored closely and considered for euthanasia
3. The smallest volume of priming agent (e.g., 0.1-0.5 ml Pristane) necessary to elicit the growth of ascitic tumors should be used. This practice also reduces potential distress caused by the irritant properties of the priming agent.
4. Hybridomas should be tested for adventitious infectious agents before introduction into the animal host to prevent potential transmission of these infectious agents from contaminated cell lines into facility mouse colonies and possibly to humans handling the animals.
5. Usually 10^5 - 10^7 cells in 0.1-0.5 ml of sterile media are inoculated 10-14 days after priming. Generally, very high concentrations are associated with greater mortality and concentrations $< 1 \times 10^5$ cells elicits fewer ascitic tumors and have a smaller volume yield.
6. Ascites pressure should be relieved before abdominal distension is great enough to cause discomfort or interfere with normal activity.
 - a. Manual restraint or anesthesia may be used for tapping.
 - b. Aseptic technique should be used in withdrawing ascitic fluid.

¹ Approved by the IACUC on: September 20, 2001, reviewed 9/25/12, 4/27/18, 1/15/21, 1/18/24

² Monoclonal Antibody Production. NRC. National Academy Press, Washington, DC 1999.

- c. The smallest needle possible that allows for good flow should be used (18-22 guage).
7. Animal(s) should be monitored closely following the tap to observe possible signs of shock due to fluid withdrawal:
 - a. Pale eyes, ears and muzzle and breathing difficulties are indicative of circulatory shock.
 - b. Shock may be prevented or treated with 2-3 ml warm saline or lactated ringers administered subcutaneously or intraperitoneally.
8. The number of taps should be limited, based on good body condition of the animal. Two survival taps (the 3rd being terminal) are recommended. Additional taps should have individual IACUC approval.
9. Animals should be monitored at least once daily, 7 days a week by personnel familiar with clinical signs associated with ascites production and circulatory shock.

Animals should be euthanized appropriately before the final tap or at any point if there is evidence of debilitation, pain or distress. Signs of distress include hunched posture, rough haircoat, reduced food consumption, emaciation, inactivity, difficulty ambulating, respiratory problems, and solid tumor growth.