

**Arizona State University
Institutional Animal Care and Use Committee
STANDARD INSTITUTIONAL GUIDELINE**

ANTIBODY PRODUCTION IN RABBITS

Polyclonal Antibody Production in Rabbits

Polyclonal antisera are universally accepted as reagents for a variety of detection assays. Rabbits are often the preferred species in which to develop the antibodies because rabbits (1) have peripheral veins and arteries that are relatively easy to access, (2) allow for the regular and uneventful collection of adequate volumes of blood, and (3) have well-defined husbandry methods.

While most rabbits produce exceptional titers to the injected antigen, this is not always the case. Thus, protocols may require more than one rabbit for the production of a single antibody. To reduce the number of animals used, it is recommended to analyze pre-immune serum of a single rabbit for cross-reactivity prior to ordering additional animals. Some animals yield high titers of antiserum, therefore, only one animal may be necessary. With proper care and occasional antigen booster injections, a rabbit can successfully produce antibodies for a few years, if necessary.

Adjuvant Use

Adjuvants greatly enhance the magnitude and duration of antibody response, and promote the more desirable secondary (IgG) response. Traditionally, Freund's Complete Adjuvant has been used for the initial antigen injection with booster injections limited to Freund's Incomplete Adjuvant. While Freund's adjuvants are frequently used successfully in rabbits, we recommend investigators consider newer, alternative adjuvants (e.g., TiterMax, Quil-A or Ribi). Unless noted in the IACUC protocol, the maximum total antigen injection volume is 1.0 ml, but not to exceed more than 0.5ml at any one site.

Rabbit Acquisition and Possible Screening

Rabbits must be purchased from commercial vendors through the Department of Animal Care and Technologies. To obtain blood samples, either for pre-immune serum or subsequent follow up titers, the rabbit can be restrained in either a towel or commercial restrainer, and the fur is shaved from one of the ears. The skin is washed using betadine, chlorhexidine, or other disinfectant. A small gauge butterfly syringe or needle and syringe is inserted into the marginal vein of the ear and up to 3 ml of blood is drawn back slowly into a syringe. The needle is removed and pressure is applied to prevent bruising. The rabbit should be observed briefly after returning to its cage to ensure there is no additional bleeding caused by the rabbit shaking its head or scratching the ear.

Antigen Injection and Blood Collection Schedule

To minimize negative side effects, investigators should use aseptic conditions, select the proper site of injection, and inject the smallest volume possible. Regardless of route of delivery, the inoculum should be as contaminant-free as possible (i.e., filter through a 0.2 micron filter) and adjusted to a biologic pH to reduce the likelihood of an excessive inflammatory response. The rabbit is restrained as above, a patch of skin on the left cranial quadrant of the back may be shaved and prepped with chlorhexidine or alternate disinfectant, and the antigen is injected subcutaneously (a maximum of 0.5 ml of injectate is

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injected into a single location). Booster injections are given in a similar fashion but in different locations approximately every two to three weeks until an adequate titer is achieved. The first, second, and third booster injections are given in the right cranial, right caudal, and left caudal quadrants of the back, respectively. Additional booster injections, if necessary, are rotated through the four regions of the back in a clockwise manner. Using this clockwise quadrant approach helps in assessing the time of any problematic antigen injections. A post-immunization blood sample (maximum of 3 ml) may be collected as described above within one week of each booster injection to assess serum titer. Once an adequate titer is obtained, typically a terminal blood collection is performed to harvest antibodies. This is done by first deeply anesthetizing the rabbit. Once a surgical plane is obtained and verified through lack of ocular and pedal reflexes, the rabbit is exsanguinated via cardiocentesis followed by a secondary thoracotomy. Alternatively to a terminal bleed, antibodies can be harvested via non-terminal blood collection (maximum 1.0% of body mass) no more frequently than monthly.

The following is a sample schedule for antigen injections and blood collection. However, the number of injections, number of blood draws, and exact days between events may vary within the limits described above.

Day 0 - pre-immunization sample (not to exceed 3 ml)

Day 1 - initial antigen injection (maximum 1.0 ml, divided 0.5 ml per injection site)

Day 15 - 2nd antigen injection (same limits as initial injection)

Day 21 - blood sample for antibody titer (not to exceed 3 ml)

Day 30 - 3rd antigen injection (same limits as initial injection)

Day 45 - blood sample for antibody titer (not to exceed 3 ml) Results of this test may indicate the need for additional boosts if the titer is not sufficient. Caution needs to be taken with additional boosts so that the rabbit does not become tolerized. A good rule of thumb is an upper limit titer of 1:100,000. This may not be attainable depending on the immunogenicity of the protein.

Day 50+ (depending on titer) - an additional boost may be indicated (same limits as initial injection)

Day 50*+ - terminal blood collection to harvest antibodies. Alternatively, a blood sample not exceeding 1% body mass can be collected as frequently as monthly.

* A final blood sample prior to terminal collection is recommended.

Potential Clinical Impact

Using the above described procedures, clinical concerns are uncommon. One of the more likely negative impacts is abscess development at the site of injection. Such abscesses may be sterile or infectious. Most abscesses are self-limiting, maintaining a relatively small size (< 1 cm diameter) with no discoloration or ulceration of the skin. If abscesses become larger or ulcerate, the DACT veterinary team is consulted and treatment follows their recommendation. Possible treatments include lancing and flushing the abscess, topical and or systemic antibiotics, and surgical removal of the abscess. In cases where abscesses form, regardless of size, the DACT veterinary team will review with the laboratory and technical staff the procedures used for antigen preparation, purification, and injection. Oftentimes, modification of technique (e.g., change adjuvant, enhance purification methods) can prevent abscess formation from future antigen injections.

Protocol Requirements for using this SIG

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This SIG is not a stand-alone protocol. Rather, investigators can refer to it in their IACUC Animal Use Protocol. In addition to referencing this SOP, investigators must include the following procedural information in their Animal Use Protocol:

- How the antigen is prepared and purified (i.e., what methods were used to remove impurities and what impurities likely remain), and what adjuvants will be used.
- Who will be injecting the antigen and collecting the blood samples.
- The maximum duration that the rabbit will be used to produce antibodies.