

Producing Polyclonal Antibodies in Rabbits

Updated by UCAR 12/15/21

The following general guidelines have been developed by UCAR for investigators who plan to produce polyclonal antibodies in rabbits.

Injection Sites and Volumes:

1. The hair at the injection site(s) must be clipped. The skin must be wiped with 70% alcohol or another antiseptic.
2. Intradermal route: A sterile 25 or 26 gauge needle is used for each injection, not to exceed a volume of 0.025 ml (25 microliters) per site¹. The location of the site(s) is over the dorsal thoracic or lumbar region (avoiding the scruff) bilaterally, spaced 3-4 cm away from each other to prevent confluence.
3. Subcutaneous route: A sterile 22 to 26 gauge needle is used for each injection not to exceed 0.25 ml (250 microliters) per site over the dorsal thoracic and lumbar region (avoiding the scruff) spaced 3-4 cm from each other.
4. Intramuscular route: A sterile 22 to 26 gauge needle is used for each injection not to exceed 0.25 ml (250 microliters) per site in the biceps femoris and not to exceed one injection per thigh.

Any complications or adverse effects associated with immunizations (e.g. swelling, drainage, ulceration) must be reported to DCM.

Adjuvants:

- Complete Freund's adjuvant may only be used at the initial immunization. Incomplete Freund's adjuvant may be used at subsequent booster immunizations.
- Other adjuvants are available. Contact the DCM for information.

Blood Collection:

1. Sedation is recommended for blood collection from the central ear artery. Sedation with acepromazine 1mg/kg SC or IM provides chemical restraint and vasodilates the central ear artery making blood collection easy to accomplish. A rabbit restrainer is required to protect the rabbit from injuring its back. DCM staff may provide more information on sedation.
2. Exsanguination is performed under a surgical plane of anesthesia (ketamine 44 mg/kg IM plus xylazine 5 mg/kg IM or other appropriate anesthetic) via intracardiac route. Withdrawal reflexes must be assessed to confirm the animal is at an appropriate anesthetic plane prior to exsanguination.

Blood volumes: No more than 10-15% of the rabbit's blood volume (6-9 ml/kg bw.) may be drawn at one time for a survival procedure. If more blood must be drawn in a survival procedure, UCAR must be contacted for justification and DCM must monitor the packed cell volume (PCV) and body weight trends and evaluate for fluid replacement. Blood may not be collected more than once every two weeks.

¹ Halliday et al. (2000) "Physiologic and Behavioral Assessment of Rabbits Immunized with Freund's Complete Adjuvant". Contemporary Topics 39(5) 8-13.