

PRODUCTION OF RABBIT ANTISERA

10 week old female New Zealand white rabbits can be ordered from Gibson & Simonsen or Animal Care Facilities and before immunization can be bled for normal control serum. About 3 rabbits per antigen is advisable in case of death. Animals should be given a few days to settle into the new environment. Any premature deaths will occur in this period. The immunization protocol is as follows:

When using purified antigens (e.g. Myoloma) use 100 µg in 1 ml saline mixed with 3 ml complete Freund's. For conjugates (e.g. haptens) use 1 ml of 2 mg/ml solution mixed with 3 ml complete Freund's. Use two-way needle to homogenize the protein-Freund's mixture. Vols. may be measured directly into the syringes.

Injections Sites	~ Volume
The four foot pads (rabbit tied down)	0.3 ml per site (1.2 ml)
The ventral surface - four lymph nodes (rabbit tied down) (armpits & groin) draining the arms and legs	0.3 ml “ “
The dorsal surface - four lymph nodes (as above)	0.3 ml “ “
Intraperitoneally (rabbit tied down)	0.4 ml “ “
13 sites	4.0 ml/rabbit

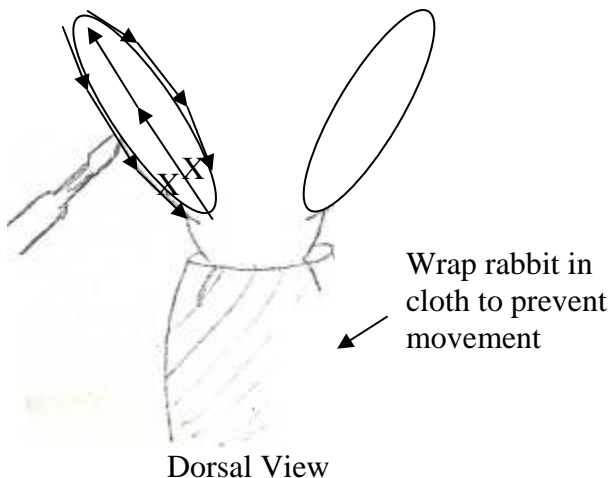
Use 1 1/2" x 22 gauge needle.

3 to 4 weeks are allowed to pass before the boost injection is given. The boost consists of 0.5 ml of the 2 mg/ml conjugate or 0.5 ml containing 100 µg purified antigen mixed with 0.5 ml of Complete Freund's Adjuvant using the two-way needles.

Injection Site	Volume
Intramuscular in the hip	1 ml/rabbit

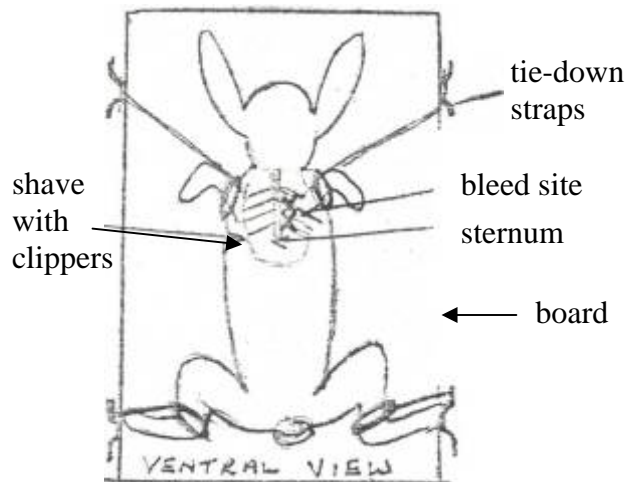
Wait 5 to 7 days before bleeding the rabbit.

For small samples of blood for testing. Bleed from the ear. Use vaseline to mat hair and stop blood clotting. Rub the ear to improve circulation (a small touch of xylene at the tip of the ear helps).



Then nick vein with sharp pointed scalpel blade and clamp off the Veins by finger pressure at the points marked X. Collect blood into tube. When bleed is completed release finger pressure and cool ear by soaking with alcohol. Wash tip of ear well with alcohol if Xylene has been used. A dab of Vaseline on the cut will prevent accidental bleeding and help healing.

For Production Bleeds: -- use heart puncture.



A 50 ml syringe and 19 gauge x 1 1/2" needle is used. Trace down the centre of the rib cage with finger until sternum' is reached. Now enter rib cage between bottom and 2nd from bottom or between latter and 3rd from bottom rib just off centre to the right. Apply vacuum to syringe and proceed slowly downwards toward centre of rib cage. As soon as blood enters syringe hold syringe and needle absolutely steady so as not to lose the site. Collect 45-50 ml of blood. Remove needle from syringe, to empty blood into centrifuge tube, gently so as not to cause hemolysis. Make sure rabbit has plenty of drinking water.

Allow blood to clot. Free clot from glass tube with spatula or pasteur pipet and allow clot to shrink to about half its original size (~4 hours at room temp or overnight at +4°C or combination of both).

Remove clot from tube with spatula and centrifuge tube at 2,000 rpm for 10 min. Collect serum with Pasteur pipet and place in clean bottle making sure no free/loose red cells are picked up, stopper and freeze.

Ammonium sulphate precipitation

Saturated solution of $(\text{NH}_4)_2\text{SO}_4$ in distilled water stored at +4°C. Add SAS (saturated ammonium sulfate) to serum dropwise with continuous stirring at +4°C to give you a 40% saturated ammonium sulphate precipitation.

$$* \text{ volume of SAS needed} = \frac{\text{ml serum to be precipitated} \times 4}{6}$$

Allow to stand at +4°C for 1 hour. Centrifuge at 10,000 rpm for 15 min. Remove supernatant (do not discard until ppt has been tested). Resuspend ppt in 1 x PBS (4) as small a volume as possible, transfer to a dialysis bag including a rinse of the centrifuge tube. Dialyse overnight against 1x PBS pH 7.0 with 0.1% NaN_3 . Empty bag into tube including a rinse of the bag with 1x PBS. Stopper and freeze.

- (1) Freund's Adjuvant Incomplete may be obtained from Difco Laboratories.
- (2) Syringes and needles are all disposable.
- (3) Two-way needle (not disposable) [16 gauge, stainless steel needle, over a 1" long brass base]
- (4) 10x pbs Phosphate buffered saline (Dilute to 1x with dist. Water before use)

For 1 litre of 10x

NaCl	76.5 gm
Na_2HPO_4 (anhydrous)	7.25 gm
KH_2PO_4	2.12 gm
NaN_3	10.0 gm

Make up to 1 litre with dist. Water. Adjust pH to 6.5 with in HCl when diluted to 1x pH will be 7.0 - 7.1.

Note: Add sodium azide just prior to making up to volume.