

Standard Sheep Polyclonal Antibody Protocol for Protein and Peptide Antigens

Subject	Dorsett Sheep, Preconditioned, Q Fever Negative
Adjuvant	Complete Freund's Adjuvant (CFA) initially, followed by Incomplete Freund's Adjuvant (IFA) for all subsequent injections.
Immunogen	Typically proteins and conjugated peptides. Typically 50 to 500 µg immunogen is used for each immunization (however amounts may vary do to antigen availability).
Procedure	The immunogen is diluted to 0.25 ml with sterile saline and combined with 0.25 ml of the appropriate adjuvant. The antigen and adjuvant are mixed thoroughly to form a stable emulsion that is injected intramuscularly (IM) into the hind quadriceps of the sheep and/or intradermally into the hind haunches near the rear legs for a maximum of two times intradermally. This provides enhanced immune response from the sustained presentation of the immunogen. PRF&L has established immunization SOPs and Guidelines to be followed.
Bleeds	Blood is collected from the jugular vein with a 16-gauge needle and allowed to clot and retract at 37°C overnight. The serum is decanted and clarified by centrifugation at 2,500 RPM for 15 minutes. PRF&L has established blood collection and laboratory SOPs and Guidelines to be followed.
Schedule	<p>Day 0 Preimmune Bleed amount depending upon request Large Preimmune yields about 125 -- 200 ml of sera (weight dependent) or Small Preimmune yields about 60 ml sera Test Preimmune yields 3 – 5 ml sera Immunize with 100 -- 500 µg/sheep antigen in CFA intradermally. (if concentration of the antigen is less than 0.8mg/ml also inject subcutaneous or IM)</p> <p>Day 14 Immunize with 50 --100 µg/sheep antigen in IFA intradermally. (if concentration of the antigen is less than 0.8mg/ml also inject subcutaneous or IM)</p> <p>Day 28 Immunize with 50 --100 µg/sheep antigen in IFA intramuscularly.</p> <p>Day 42 Small or Test Bleed</p> <p>Day 44 Ship preimmune and small bleed serum to project sponsor for testing or PRF&L can perform ELISAs at the sponsor's request</p> <p>Day 56 Immunize with 25 -- 50 µg/sheep antigen in IFA intramuscularly.</p> <p>Day 70 Small or Test Bleed, continuing every 28 days</p> <p>Day 72 Ship serum to project sponsor for testing or PRF&L can perform ELISAs at the sponsor's request. The project can be discussed with PRF&L Staff and alterations to the schedule may be implemented at this point.</p> <p>Day 84 Immunize with 25 -- 50 µg/sheep antigen in IFA intramuscularly and continuing every 28 days.</p>
Continuing	The sheep is maintained on a four-week cycle of immunizations and bleeds. The results of testing will determine whether the protocol should be continued, modified, or terminated. Extra weekly production large bleeds and immunizations can be performed upon sponsor's request providing they fall within PRF&L Guidelines and SOPs.
Endpoint	The endpoint of the project is determined by the feedback received from the sponsor. He/she may request to terminate the project with serum (by exsanguination) or without serum (humanely euthanize or discard). PRF&L reserves the right to terminate a sheep prematurely that shows signs of morbidity.