



Quality Antibody Production Since 1960

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Standard Rabbit Polyclonal Antibody Protocol for Fusion Protein Antigens

Subject	SPF New Zealand White Female Rabbit
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 200 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 beneath the skin of the rabbit (subcutaneously) in the area around the shoulders 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 central ear artery with a 21-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 15 -- 20 ml of sera or Small Preimmune yields about 3 – 5 ml sera Immunize with 100 -- 200 µg/rabbit antigen in CFA intradermally. (if concentration of the antigen is less than 0.8mg/ml also inject subcutaneous)</p> <p>Day 28 Immunize with 50 --100 µg/rabbit antigen in IFA intradermally. (if concentration of the antigen is less than 0.8mg/ml also inject subcutaneous)</p> <p>Day 42 Immunize with 50 --100 µg/rabbit antigen in IFA subcutaneous.</p> <p>Day 56 Small Bleed (3 – 5 ml sera) Immunize with 25 -- 50 µg/rabbit antigen in IFA subcutaneous. Immunize with 25 -- 50 µg/rabbit antigen intravenous (no adjuvant).</p> <p>Day 63 Large Bleed (10 – 15 ml sera)</p> <p>Day 65 Ship preimmune, small bleed and large bleed 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 70 Immunize with 25 -- 50 µg/rabbit antigen in IFA subcutaneous and continuing every 28 days.</p> <p>Day 84 Small Bleed (3 – 5 ml sera) ,continuing every 28 days.</p>
Continuing	The rabbit 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 rabbit prematurely that shows signs of morbidity.