

## Standard Rat Polyclonal Antibody Protocol for Fusion Protein Antigens

<b>Subject</b>	VAF Long Evans Female Rat
<b>Adjuvant</b>	Complete Freund's Adjuvant (CFA) initially, followed by Incomplete Freund's Adjuvant (IFA) for all subsequent injections.
<b>Immunogen</b>	Typically proteins and conjugated peptides. Typically 10 to 100 µg immunogen is used for each immunization (however amounts may vary do to antigen availability).
<b>Procedure</b>	The immunogen is diluted to 0.125 ml with sterile saline and combined with 0.125 ml of the appropriate adjuvant. The antigen and adjuvant are mixed thoroughly to form a stable emulsion that is injected intraperitoneally in the stomach area. This provides enhanced immune response from the sustained presentation of the immunogen. PRF&L has established immunization SOPs and Guidelines to be followed.
<b>Bleeds</b>	Blood is collected from the retro-orbital sinus by a microhematocrit tube into heparin. The plasma 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.
<b>Schedule</b>	<p><b>Day 0</b> Preimmune Bleed yields about 0.5 ml of plasma. Immunize with 50 -- 100 µg/rat antigen in CFA intraperitoneally. (if concentration of the antigen is less than 0.8mg/ml also inject subcutaneous)</p> <p><b>Day 28</b> Immunize with 25 --50 µg/rat antigen in IFA intraperitoneally. (if concentration of the antigen is less than 0.8mg/ml also inject subcutaneous)</p> <p><b>Day 42</b> Immunize with 25 --50 µg/rat antigen in IFA interperitoneally.</p> <p><b>Day 56</b> Bleed (0.5 ml plasma) Immunize with 25 -- 50 µg/rat antigen in IFA interperitoneally. Immunize with 25 -- 50 µg/rat antigen intravenous (no adjuvant).</p> <p><b>Day 63</b> Bleed (0.5 ml plasma)</p> <p><b>Day 65</b> Ship preimmune and the two bleed plasma to project sponsor for testing or PRF&amp;L can perform ELISAs at the sponsor's request. The project can be discussed with PRF&amp;L Staff and alterations to the schedule may be implemented at this point.</p> <p><b>Day 70</b> Immunize with 25 -- 50 µg/rat antigen in IFA interperitoneally and continuing every 28 days.</p> <p><b>Day 84</b> Bleed (0.5 ml plasma), continuing every 28 days.</p>
<b>Continuing</b>	The rat 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 bleeds and immunizations can be performed upon sponsor's request providing they fall within PRF&L Guidelines and SOPs.
<b>Endpoint</b>	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 rat prematurely that shows signs of morbidity.