

## Standard Chicken Polyclonal Antibody Protocol for Fusion Protein Antigens

<b>Subject</b>	Sex-Sal or Leghorn Hen
<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 50 to 200 µg immunogen is used for each immunization (however amounts may vary do to antigen availability).
<b>Procedure</b>	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 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 brachial vein with an 18-gauge needle and allowed to flow into a tube with 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 about 10 ml of plasma; also collect preimmune eggs if requested. Immunize with 100 -- 200 µg/chicken antigen in CFA intramuscularly.</p> <p><b>Day 28</b> Immunize with 50 --100 µg/chicken antigen in IFA intramuscularly.</p> <p><b>Day 42</b> Immunize with 50 --100 µg/chicken antigen in IFA intramuscularly.</p> <p><b>Day 56</b> Bleed (10 ml plasma) Immunize with 25 -- 50 µg/chicken antigen in IFA intramuscularly. Immunize with 25 -- 50 µg/chicken antigen intravenous (no adjuvant).</p> <p><b>Day 63</b> Bleed (10ml plasma)</p> <p><b>Day 65</b> Ship preimmune, 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/chicken antigen in IFA intramuscularly and continuing every 28 days.</p> <p><b>Day 84</b> Bleed (10 ml plasma), continuing every 28 days.</p>
<b>Continuing</b>	The chicken 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. As an added benefit, chicken eggs can be collected to isolate the IgY.
<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 plasma (by exsanguination) or without plasma (humanely euthanize or discard). PRF&L reserves the right to terminate a chicken prematurely that shows signs of morbidity.