



**ABGENT CUSTOM SERVICES:
RABBIT POLYCLONAL ANTIBODY
PRODUCTION PROTOCOL**

(Rev.: 02-20-2009, PP)

1. Subject	New Zealand Rabbits.
2. Adjuvant	Complete Freund's Adjuvant (CFA) initially, followed by Incomplete Freund's Adjuvant (IFA) for all subsequent injections.
3. Immunogen	KLH conjugated phospho-peptide. 50-100 µg immunogen is used for each immunization.
4. Procedure	The immunogen is diluted to 0.5 ml with sterile saline and combined with 0.5 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 intra-muscularly into the large muscle of the rear legs. (That is about 1/4 of the antigen being used in each area). This provides enhanced immune response from the sustained presentation of the immunogen.
5. Bleeds	Blood is collected from the central ear artery with a 19-gauge needle and allowed to clot and retract at 37°C overnight. The clotted blood is then refrigerated for 24 hours before the serum is decanted and clarified by centrifugation at 2,500 RPM for 20 minutes.
6. Schedule	<p>Week 0 Bleed 10 ml (yields 2-3 ml pre-immune serum) Immunize with 200 µg/rabbit antigen in CFA.</p> <p>Week 2 Immunize with 100 µg/rabbit antigen in CFA.</p> <p>Week 4 Immunize with 100 µg/rabbit antigen in IFA.</p> <p>Week 5 Test bleed 10-20 ml/rabbit. ELISA on appropriate cell/tissue.</p> <p>Week 6 Immunize with 100 µg/rabbit antigen in PBS. 1st Production bleed 20-30 ml if test positive. Immunize with 100 µg/rabbit antigen in PBS. ELISA test again if previous tests were negative.</p> <p>Week 7 2nd Production bleed 20-30 ml. Immunize with 100 µg/rabbit antigen in PBS. ELISA test again if previous tests were negative. The continuation of the project needs to be discussed if negative.</p> <p>Week 8 3rd Production bleed 20-30 ml. Immunize with 100 µg/rabbit antigen in PBS.</p> <p>Week 9 4th Production bleed 20-30 ml. Immunize with 100 µg/rabbit antigen in PBS.</p> <p>Week 10 Terminating bleed 30-50 ml/rabbit. Protein A purification of pooled bleeds. Average yield of purified Ig fraction is 100-150 mgs.</p>
7. Note	The rabbits can be maintained on a ten-weeks cycle of immunizations and bleeds as required by the project. The results of testing will determine whether the program should be continued, modified, or terminated. Extra bleeds can be taken upon customer's requirement of extended project with extra charge.