

Polyclonal Production Protocol

Rat Protein Schedule of Events, 118 Day

Day:	Procedure:
0	Rat Pre-bleed (Avg. 0.5 ml serum) 1° SC: 200 micrograms with FCA
21	Boost SC: 100 micrograms with FIA
31	Test Bleed (Avg. 0.5 ml serum)
42	Boost SC: 100 micrograms with FIA
52	Production Bleed (Avg. 2.0 ml serum)
53	ELISA Titer Assay of Bleed *
63	Boost SC: 100 micrograms with FIA
73	Production Bleed (Avg. 2.0 ml serum)
84	Boost SC: 50 micrograms with FIA
94	Production Bleed (Avg. 2.0 ml serum)
95	ELISA Titer Assay of Bleed *
105	Boost SC: 50 micrograms with FIA
115	Production Bleed (Avg. 2.0 ml serum)
118	Terminal bleed (Avg. 7.0 ml serum)

Total material required for injection for the above program is 600 micrograms/animal.

If we are conjugating protein for you we assume a 20% loss of protein during conjugation.

Total amount of serum expected from a mouse on the above schedule of events is approximately 15.5 ml.

Please Note:

- It is recommended that a minimum of three rats be used due to biological variations.
- Immunogen is emulsified in Freund's Complete Adjuvant (FCA) for initial injections. Freund's Incomplete Adjuvant (FIA) is used for all subsequent injections (boosts). Projects follow a three-week cycle of boosts. Test bleeds are taken approximately 10 days after the boosts.

***Optional ELISA:** We suggest testing the first bleed of the project to evaluate the effect of antigen on the animal. Information gained from an ELISA at this point will provide an opportunity for you to make adjustments to your immunization schedule. Additional ELISA's performed later in the project will give you valuable information that can be used to determine which sera samples to include in your research or which bleeds you would like to have purified.