

Polyclonal Production Protocol

Chicken Protein Schedule of Events, 118 Day

Day:	Procedure:
0	Female Chicken Pre-bleed (Avg. 2 ml serum) 1° IM/SC: 250 micrograms with FCA
21	Boost IM/SC: 125 micrograms with FIA
35	Test Bleed (Avg. 2 ml serum)
42	Boost IM/SC: 125 micrograms with FIA
56	Test Bleed (Avg. 2 ml serum)
57-63	ELISA Titer Assay of Bleed *
63	Boost IM/SC: 125 micrograms with FIA
77	Test Bleed (Avg. 2 ml serum)
78-84	ELISA Titer Assay of Bleed *
84	Boost IM/SC: 125 micrograms with FIA
98	Test Bleed (Avg. 2 ml serum)
105	Boost IM/SC: 125 micrograms with FIA
115	Test Bleed (Avg. 2 ml serum)
118	Terminate with Bleed (Avg. 10 ml serum)

Total material required for injection for the above program is 875 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 chicken on the above schedule of events is approximately 18 ml. The bulk of the antibody however, will be obtained from the eggs.

Please Note:

- Egg collection is performed at no charge. However, the investigator must inform Covance when egg collection is to be initiated.
- It is recommended that a minimum of two chickens 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 eggs you would like to have purified.