



Hooke Laboratories

## Immunization of Mice for Production of Antigen-Specific Antibodies

Recommended protocol for use with:

- Hooke Kit™ Ovalbumin/CFA Emulsion (EK-0301)
- Hooke Kit™ Ovalbumin/IFA Emulsion (EK-0311)

### Summary

This protocol describes immunization of mice to induce production of high concentrations of antibodies specific for an antigen.

Mice are immunized with protein emulsified in complete Freund's adjuvant (CFA), followed by one or two booster doses of protein emulsified in incomplete Freund's adjuvant (IFA).

### Materials needed

Qty	Description
1 to 2	Hooke Kit™ Ovalbumin/CFA Emulsion (EK-0301), and
1	Hooke Kit™ Ovalbumin/IFA Emulsion (EK-0311)
20	Laboratory mice (any strain, males or females)

### Protocol

Mice should be acclimated at your facility for at least 7 days before immunization.

Inject mice with antigen emulsified in CFA subcutaneously at two sites on the back, injecting 0.05 to 0.1 mL at each site (total of 0.1 to 0.2 mL per mouse). A stronger immune response is generated by injecting at two sites and in larger volumes.

Keep the needle inserted into the subcutaneous space for 10 to 15 seconds after each injection to avoid leakage of the emulsion. Alternatively, a light pull on the syringe plunger will prevent leakage.

A booster injection of antigen emulsified in IFA is administered 10 to 20 days (14 days recommended) after immunization with antigen/CFA emulsion. The booster is given as a single subcutaneous injection with 0.1 mL of IFA emulsion, at one site on the back.

A serum sample is isolated from one or more mice 7 to 10 days after the first booster dose, and antibody concentration is tested. If the antibody concentration is less than desired, an additional booster dose of IFA emulsion may be given 10 to 20 days (14 days recommended) after the first booster dose.

Serum is typically collected 7 to 14 days after administration of the last booster dose.

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