



Hooke Laboratories

Immunization of Mice with Protein

Recommended protocol for use with:

- Hooke Kit™ Ovalbumin/CFA Emulsion (EK-0301)
- Hooke Kit™ Pigeon Cytochrome c/CFA Emulsion (EK-0302)
- Hooke Kit™ Hen Egg Lysozyme/CFA Emulsion (EK-0303)
- Hooke Kit™ Ovalbumin/IFA Emulsion (EK-0311)
- Hooke Kit™ Pigeon Cytochrome c/IFA Emulsion (EK-0312)
- Hooke Kit™ Hen Egg Lysozyme/IFA Emulsion (EK-0313)

Summary

This protocol may be used either to test immune responses in mice¹ or to induce production of antibodies specific to an antigen.

To test immune responses, mice are immunized with protein emulsified in Complete Freund's Adjuvant (CFA). Separate procedures are provided for:

- evaluation of immune responses in inguinal lymph nodes or spleen,
- inducing delayed-type hypersensitivity (DTH), and,
- evaluating antibody response

To induce production of high concentrations of antibodies specific to the administered antigen, mice are immunized with protein emulsified in CFA, followed by one or two booster doses of protein emulsified in Incomplete Freund's Adjuvant (IFA).

Materials needed

To test immune responses:

Qty	Description
1	Hooke Kit™ Ovalbumin/CFA Emulsion (EK-0301), or, Hooke Kit™ Pigeon Cytochrome c/CFA Emulsion (EK-0302), or, Hooke Kit™ Hen Egg Lysozyme/CFA Emulsion (EK-0303)
10 to 20	Laboratory mice (any strain, males or females)

¹ Product can also be used with rats and other species. This protocol addresses mice only.

To induce high concentrations of antibody production:

Qty	Description
1 to 2 1	Hooke Kit™ Ovalbumin/CFA Emulsion (EK-0301), and Hooke Kit™ Ovalbumin/IFA Emulsion (EK-0311), or,
1 to 2 1	Hooke Kit™ Pigeon Cytochrome c/CFA Emulsion (EK-0302), and Hooke Kit™ Pigeon Cytochrome c/IFA Emulsion (EK-0312), or,
1 to 2 1	Hooke Kit™ Hen Egg Lysozyme/CFA Emulsion (EK-0303), and Hooke Kit™ Hen Egg Lysozyme/IFA Emulsion (EK-0313)
20	Laboratory mice (any strain, males or females)

Evaluation of immune responses in inguinal lymph nodes or spleen -- Method

Mice should be acclimated at your facility for at least 4 days before immunization.

Inject mice with antigen emulsified in CFA subcutaneously at two locations in the base of the tail (one on each side of the tail base), depositing the emulsion at the hair line. Only 0.05 ml/injection site should be administered.

Immune response is usually evaluated 10 to 14 days after immunization by looking at antigen-specific proliferation and cytokine production of the draining lymph nodes and/or spleen cells.

Evaluation of immune response depends on experimental design.

Inducing delayed-type hypersensitivity (DTH) -- Method

Mice should be acclimated at your facility for at least 4 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.

For evaluation of DTH, 5 to 10 days after immunization mice are challenged with antigen in phosphate buffered saline (PBS) (not supplied). Typically, antigen in PBS is injected into footpad or ear and the counter-lateral footpad or ear is injected with only PBS, as a negative control.

Evaluation of antibody response - Method

Mice should be acclimated at your facility for at least 4 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.

Serum is typically collected 14 days after immunization.

Production of high concentrations of specific antibodies - Method

Mice should be acclimated at your facility for at least 4 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 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 10 days after administration of the last booster dose.

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