

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)

Summary

Mice¹ are immunized with protein emulsified in Complete Freund's Adjuvant (CFA) to test their immune response.

Materials needed

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)

Method

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

The mice can be injected either in the base of the tail or in the back. Injection at base of tail is preferred when evaluating immune responses in draining inguinal lymph nodes. Mice are usually injected in the back when inducing delayed-type hypersensitivity (DTH) or evaluating antibody response.

Evaluation of immune response depends on experimental design.

Base of tail injection - evaluation of immune responses

Inject mice 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.

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

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.

Back injection

Inject mice with the emulsion 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.

Evaluation of DTH or antibody response

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.

For evaluation of antibody response, serum is typically collected 14 days after immunization.

Version: 2009-08