Hooke Laboratories

Immunization of C57BL/6 Mice with MOG_{35-55}

Recommended protocol for use with Hooke Kit™ MOG_{35-55}/CFA Emulsion (EK-0111).

Summary

C57BL/6 mice are immunized with MOG_{35-55} peptide emulsified in Complete Freund's Adjuvant (CFA) either to test their immune response to this peptide or to generate encephalitogenic cells for adoptive transfer EAE. Most mice will not develop EAE after immunization with MOG_{35-55} /CFA. If EAE develops, it will be milder than when pertussis toxin is used and the onset will be significantly later than in mice injected with MOG_{35-55} /CFA and pertussis toxin.

Materials needed

Qty	Description
1	Hooke Kit™ MOG_{35-55}/CFA Emulsion (EK-0111)
10	C57BL/6 mice, females (Taconic Farms model B6-F or Jackson Laboratory strain C57BL/6J)

Method

To immunize C57BL/6 mice, use Hooke Kit™ MOG_{35-55}/CFA Emulsion (EK-0111).

Mice should be 8-16 weeks old, acclimated at your facility for at least 4 days before immunization.

The mice can be injected either in the back or at the base of the tail. Mice are usually injected in the back when generating encephalitogenic cells. Injection at base of tail is preferred when evaluating immune responses in draining inguinal lymph nodes.

Back injection – generation of encephalitogenic T cells

Inject mice with the emulsion subcutaneously, at two sites on the back, injecting 0.1 ml at each site (total of 0.2 ml of emulsion per mouse). 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.

Encephalitogenic T cells can be generated by culturing splenocytes from the immunized mice 10-12 days after immunization.

439 South Union Street
Building 2, Suite 210
Lawrence, MA 01843 USA

http://hookelabs.com

Tel +1 617 475 5114
Fax +1 617 395 1352
info@hookelabs.com

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Splenocytes need to be cultured for 3 days in the presence of MOG_{35-55}. Sometimes immunomodulators (such IL-12, IL-23, anti-interferon gamma antibodies) need to be added to the cultures to generate encephalitogenic cells.

Base of tail injection – evaluation of immune responses

Inject mice subcutaneously in the base of tail, depositing the emulsion at the hair line. Only 0.05 ml/injection site should be administered. Two injections per mouse (one on each side of the tail base) should be given.

Immune response is usually evaluated 10 to 14 days after immunization by looking at MOG_{35-55}-specific proliferation and cytokine production of the draining lymph nodes and spleen cells.

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