Immunization of C57BL/6 Mice with MOG\textsubscript{35-55}

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

Summary

C57BL/6 mice are immunized with MOG\textsubscript{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\textsubscript{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\textsubscript{35-55} /CFA and pertussis toxin.

Materials needed

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<th>Qty</th>
<th>Description</th>
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<tbody>
<tr>
<td>1</td>
<td>Hooke Kit\textsuperscript{TM} MOG\textsubscript{35-55}/CFA Emulsion (EK-0111)</td>
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<td>10</td>
<td>C57BL/6 mice, females (Taconic Farms model B6-F or Jackson Laboratory strain C57BL/6J)</td>
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Method

To immunize C57BL/6 mice, use Hooke Kit\textsuperscript{TM} MOG\textsubscript{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.
Splenocytes need to be cultured for 3 days in the presence of MOG\textsubscript{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\textsubscript{35-55}-specific proliferation and cytokine production of the draining lymph nodes and spleen cells.

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