Immunization of SJL Mice with PLP\textsubscript{139-151}

Recommended protocol for use with Hooke Kit\textsuperscript{TM} PLP\textsubscript{139-151}/CFA Emulsion (EK-0122).

Summary

SJL mice are immunized with PLP\textsubscript{139-151} 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 before day 11 after immunization.

Materials needed

<table>
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<tr>
<th>Qty</th>
<th>Description</th>
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<tbody>
<tr>
<td>1</td>
<td>Hooke Kit\textsuperscript{TM} PLP\textsubscript{139-151}/CFA Emulsion (EK-0122)</td>
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<td>10</td>
<td>SJL mice, females (Taconic Farms model SJL-F or Jackson Laboratory strain SJL/J)</td>
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Method

To immunize SJL mice, use Hooke Kit\textsuperscript{TM} PLP\textsubscript{139-151}/CFA Emulsion (EK-0122).

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 days after immunization.
Splenocytes need to be cultured for 3 days in the presence of PLP\textsubscript{139-151}. Sometimes immunomodulators (such as IL-12, IL-23, anti-interferon gamma antibodies) are 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.

PLP\textsubscript{139-151}-specific immune responses are usually evaluated 10-14 days after immunization.

Immune response is usually evaluated 10 to 14 days after immunization by looking at PLP\textsubscript{139-151}-specific proliferation and cytokine production of the draining lymph nodes and spleen cells.

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