



Hooke Laboratories

Immunization of Mice to Induce DTH Response

Recommended protocol for use with:

- Hooke Kit™ mBSA/CFA Emulsion (cat. no. EK-0133)

Summary

This protocol describes immunization of mice with mBSA emulsified in complete Freund's Adjuvant (CFA) to induce the delayed-type hypersensitivity (DTH) response in female C56BL/6 mice.

Note - This protocol may be used with other mouse strains, but we have tested it at Hooke only in C57BL/6 mice.

Materials needed

Qty	Description
1	Hooke Kit™ mBSA/CFA Emulsion (cat. no. EK-0133)
20	C57BL/6 mice, females, 9 to 14 weeks old (Taconic Farms model B6-F or Jackson Laboratory strain C57BL/6J)
	Phosphate buffered saline (PBS) (standard formulation, pH 7.4, calcium-free, magnesium-free)
	Antigen

Protocol

Mice should be acclimated at your facility for at least 7 days before immunization.

Inject mice with antigen emulsified in CFA subcutaneously at two sites in the lower back, administering 0.05 mL over each hip (total of 0.1 mL 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.

For evaluation of DTH, 5 days after immunization mice are challenged with antigen in phosphate buffered saline (PBS). Typically, antigen in PBS is injected into footpad or ear and the counter-lateral footpad or ear (20 μ L/footpad, 10 μ L/ear) is injected with only PBS, as a negative control.

The thickness of paws or ears is measured with calipers, 24 hours after the challenge.

Note – Intradermal injection (as described for BALB/c mice in other published protocols) is not necessary in C57BL/6 mice. Subcutaneous injection of mBSA/CFA is technically easier than intradermal injection and produces an excellent DTH response in C57BL/6 mice.

References

Yoshimoto T et al, Blood 95:2869 (2000)
Särnstrand B et al, J Pharmacol Exp Ther 288:1174 (1999)
Kim YS et al, J Immunol 160 :5742 (1998)
Magram J et al, Immunity 4 :471 (1996)

Version: 2014-03