Loading of CD1d/unloaded Dextramers® with lipids

This procedure has been successfully used for loading α-Galactosyl Ceramide lipid into human and mouse CD1d/unloaded Dextramers. In this protocol the CD1d monomer is offered approximately 15 molar excess of lipids during loadning, but optimal conditions and molar stoichiometry can vary between lipids.

Materials

- Lipid (e.g. α-GalCer) dissolved in DMSO at 1 mg/ml (1.2 mM)
- CD1d/unloaded Dextramer-PE or CD1d/unloaded Dextramer-APC
- 0.5 % tween-20 in PBS

Procedure for lipid loading of 10 tests of CD1d/unloaded Dextramers:

1. Heat the lipid stock solution to 80 °C for 1 min to completely dissolve it. Dilute an aliquot of lipid in 0.5 % Tween-20, PBS, to a final concentration of 0.2 mg/ml and heat it to 80 °C for 1 min to maintain the lipid completely dissolved. Cool the lipid to 30 °C.
2. Add 5 µl of the lipid to 100 µl of CD1d/unloaded Dextramer-PE tempered to 30 °C.
3. Incubate at 30 °C overnight in the dark.
4. Store the lipid loaded CD1d Dextramer at 4 °C in the dark until use.

Notes:

- Always keep MHC Dextramer stored at 2-8°C in the dark – the brown plastic vial does not sufficiently protect the reagents against light.
- PBS: 137mM NaCl, 2.7mM KCl, 4.3mM Na_2HPO_4, 1.47mM KH_2PO_4, pH 7.4
- Visit Immudex Home page for additional information and ordering.

MHC Dextramer Reagents (RUO) are for research use only.
Not for use in diagnostic procedures.