General staining procedure for CD1d Dextramer®

As for all other protocols involving light-sensitive fluorochromes, CD1d Dextramer staining should be carried out shielded from light.

1. Transfer 1-3 x 10^6 lymphoid cells (PBMC or splenocytes) to a 12 x 75 mm polystyrene test tube. *Allocate only 2-5 x 10^5 cells per tube when staining T-cell clones or cell lines due to the high frequency of antigen-specific T cells.*
2. Add 2 ml PBS containing 5% fetal calf serum, pH 7.4. Centrifuge at 300 x g for 5 min. Remove supernatant and resuspend cells in a total volume of 50 µl PBS containing 5% fetal calf serum, pH 7.4.
3. Add 10 µl CD1d Dextramer and mix thoroughly. Incubate in the dark at room temperature for 10 min.
4. Add an optimal amount of anti-CD3 antibody conjugated with a relevant flourochrome. Additional antibodies (e.g. anti-CD8, anti-CD4, anti-CD19 and anti-CD14 antibodies) conjugated with other relevant fluorochromes may also be added at this step in appropriate amounts as recommended by manufacturer. Incubate in the dark at 2-8°C for 20 min.
5. Add 2 ml PBS containing 5% fetal calf serum, pH 7.4. Centrifuge at 300 x g for 5 min. and remove the supernatant.
6. Repeat step 5.
7. Resuspend pellet in an appropriate amount of fluid for flow cytometry, e.g. 0.4 ml PBS, pH 7.4. Analyze on a flow cytometer or store at 2-8°C in the dark until analysis. For optimal results, do not store longer than 2 hrs. before analysis.

Notes:

- Always keep CD1d Dextramer stored at 2-8°C in the dark – the brown plastic vial does not sufficiently protect the reagents against light.
- PBS: 137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, 1.47 mM KH_2PO_4
- Visit www.immudex.com for other staining procedures and "Tips and Tricks" for optimizing Dextramer staining.

CD1d Dextramer Reagents are for research use only. Not for use in diagnostic procedures.