General staining procedure for MHC II Dextramer® – PBMC's

As for all other protocols involving light-sensitive fluorochromes, MHC II 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 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 300xg 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 MHC II Dextramer to cells and mix thoroughly. Incubate in the dark at room temperature for 30 min.
4. Add an optimal amount of anti-CD4 antibody conjugated with a relevant fluorochrome. Additional antibodies (e.g. anti-CD3 or anti-CD8 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 the cell pellet in an appropriate amount of fluid for flow cytometry, e.g. 0.4 ml in 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:

- It is important that MHC II Dextramer reagent is incubated with the cells prior to addition of anti-CD4 antibody. If anti-CD4 antibody is added before or simultaneously with MHC II Dextramer, a poorer resolution of positive and negative cell populations may result.
- Always keep MHC II Dextramer stored at 2-8°C in the dark – the brown plastic vial only partially protects the reagents against light.
- Staining can be performed in 96-well plates, with use of smaller volume of PBS in step 5. However, repeated washing will then be needed. It is recommended to wash at least 5 times with 200 µl PBS containing 5% fetal calf serum, pH 7.4, each time followed by centrifugation at 300 x g for 5 min and removal of the supernatant before next washing step.
- 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 general procedures and "Tips and Tricks" for optimizing MHC Dextramer staining.

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