

## General staining procedure for MHC II Dextramer™ – PBMC's

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

1. Transfer  $1-3 \times 10^6$  lymphoid cells (PBMC or splenocytes) to a 12 x 75 mm polystyrene test tube. *Allocate only  $2 \times 10^5$  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  $\mu$ l PBS containing 5% fetal calf serum, pH 7.4.
3. Add 10  $\mu$ l 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. Add 2 ml PBS containing 5% FCS, pH 7.4, and resuspend cell pellet. Centrifuge at 300 x g for 5 min. and remove the supernatant.
7. Resuspend the cell pellet in PBS, pH 7.4, of 0.4 ml. 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.

- \* **Attention:** *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.*

### Notes:

- Always keep MHC II Dextramer stored at 2-8°C in the dark – the brown plastic vial only partially protects the reagents against light.
- PBS: 137 mM NaCl, 2.7 mM KCl, 4.3 mM Na<sub>2</sub>HPO<sub>4</sub>, 1.47 mM KH<sub>2</sub>PO<sub>4</sub>
- Visit [www.immudex.com](http://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.**