

General staining procedure MHC Dextramer™ – PBMC's

As for all other protocols involving light-sensitive fluorochromes, MHC 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 $2-5 \times 10^5$ 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 300 x g for 5 min. Remove supernatant and resuspend cells in a total volume of 50 μ l PBS containing 5% fetal calf serum, pH 7.4.
3. Add 10 μ l MHC Dextramer and mix thoroughly. Incubate in the dark at room temperature for 10 min.
4. Add an optimal amount of anti-CD8 antibody conjugated with a relevant fluorochrome. Additional antibodies (e.g. anti-CD3 or anti-CD4 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.

* **Attention:** *It is important that MHC Dextramer reagent is incubated with the cells 10 min. prior to addition of anti-CD8 antibody. If anti-CD8 antibody is added before or simultaneously with MHC Dextramer, a poorer resolution of positive and negative cell populations will result.*

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: 137 mM NaCl, 2.7 mM KCl, 4.3 mM Na₂HPO₄, 1.47 mM KH₂PO₄
- Visit www.immudex.com for staining procedures and “Tips and Tricks” for optimizing MHC Dextramer staining.

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