

Double staining with MHC Dextramer® reagents – General

Purpose: Staining Peripheral Blood Mononuclear Cells (PBMC's) with PE- and APC-labeled Dextramer reagents in the same tube.

Reagents required

MHC Dextramer/ PE
MHC Dextramer/ APC
Anti-CD8/PB antibody
Anti-CD4/FITC antibody
PBS, pH = 7.4
PBS containing 1% BSA, pH = 7.4

Due to light-sensitive fluorochromes, MHC Dextramer staining should be carried out shielded from light.

Staining protocol

1. Transfer $1-3 \times 10^6$ lymphoid cells (PBMC's) to a 12 x 75 mm polystyrene test tube. *Aliquot 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 of the same specificity.*
2. Add 2 ml PBS containing 1% BSA, 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 1% BSA, pH 7.4.
3. Add 10 μ l MHC Dextramer/PE, mix thoroughly.
4. Add 10 μ l MHC Dextramer/APC, mix thoroughly and incubate the sample in the dark at room temperature for 10 min.
5. Add appropriate amounts of anti-CD8/PB and anti-CD4/FITC antibody. Incubate in the dark at 2-8°C for 20 min.
6. Add 2 ml PBS containing 1% BSA, pH 7.4. Centrifuge at 300 x g for 5 min. and remove the supernatant.
7. Repeat step 6.
8. 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 hours 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.*

Analysis of data

The following gating is recommended for analysis of data:

- 1) Exclude irrelevant CD4⁺ cells (monocytes and T helper cells)
- 2) Exclude debris and dead cells, and include live lymphocytes
- 3) Include CD8⁺ cells
- 4) Make a PE vs. APC plot to identify CD8⁺ T cells positive for either or both of the Dextramers.

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, pH 7.4
- Visit www.immudex.com for other staining procedures and “Tips and Tricks” for optimizing MHC Dextramer staining.

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