Double staining with MHC Dextramer® reagents of same specificity

Purpose: Improve separation between the antigen-specific T cell population and the negative population, by staining with PE- and APC-labeled Dextramer reagents of the same specificity 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 x 10^6 lymphoid cells (PBMC's) to a 12 x 75 mm polystyrene test tube.
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 µl PBS containing 1% BSA, pH 7.4.
3. Just before use, add together 10 µl MHC Dextramer/PE and 10 µl MHC Dextramer/APC
4. Add the Dextramer mix to the cell suspension, and mix thoroughly.
5. Incubate the samples at room temperature, for 10 min in the dark.
6. Add appropriate amounts of anti-CD8/PB and anti-CD4/FITC antibody. Incubate in the dark at 2-8°C for 20 min.
7. Add 2 ml PBS containing 1% BSA, pH 7.4. Centrifuge at 300 x g for 5 min. and remove the supernatant.
8. Repeat step 6.
9. 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 Dextramer double positive $CD8^+$ T cells.

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 Na_2HPO_4, 1.47 mM KH_2PO_4, 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.