

Multiple MHC Dextramer™ Staining Procedure

MHC Dextramers can be pooled and added to the same sample, staining different populations of specific T cells in the same sample.

1. 3 uL of each different MHC Dextramer™ are mixed.
2. Transfer 100 µL whole blood to a 12 x 75 mm polystyrene test tube.
3. Add the MHC Dextramer™ mix to the blood sample and mix gently with a vortex mixer. Incubate in the dark at room temperature for 5 minutes.
4. Add an optimally titrated amount of a mixture of anti-CD8 and anti-CD3 antibodies conjugated with relevant fluorochromes (e.g. Dako clone DK25 and Dako clone UCHT1) and mix well. Continue incubation at 2-8 °C in the dark for 20 minutes.
5. Add 2 mL EasyLyse™ working solution (Dako Code S2364) and incubate for 10 minutes.
6. Centrifuge for 5 minutes at 300x g and aspirate supernatant.
7. Add 2 mL 0.01 mol/L PBS and centrifuge for 5 minutes at 300x g and aspirate supernatant.
8. Re-suspend pellet in an appropriate fluid for flow cytometry, e.g. 0.4 mL PBS, and analyze on a flow cytometer or store at 2-8 °C in the dark until analysis. Do not store longer than 2 hours before analysis.

Example

Staining Human Peripheral Blood Lymphocytes with Multiple MHC Dextramers

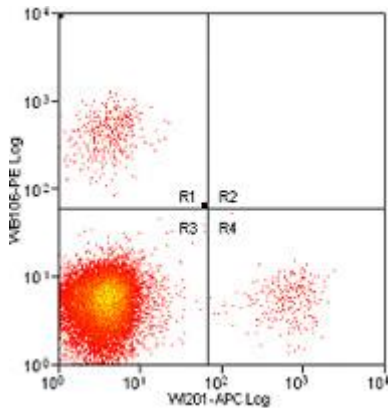


Figure A. Human peripheral blood lymphocytes purified from a blood donor were stained with mouse anti-human CD3/PB antibody, clone UCHT1 (Dako Code PB982) and the two specific MHC Dextramers HLA-A*0201(NLVPMVATV)/RPE (Immudex Code WB2132-RPE) and HLA-B*0801(ELRRKMMYM)/APC (Immudex Code WI2137-APC).

Staining was performed by mixing the two MHC Dextramers before addition to the sample, followed by addition of anti-CD3 antibody. The procedure for staining with multiple MHC Dextramers in the same sample was followed. The sample was analyzed on the CyAn™ ADP Flow Cytometer. Live-gated and CD3-positive lymphocytes are shown. 20,000 CD3-positive cells were collected.

Products and support

If you need information concerning Immudex products, have a technical enquiry, or are interested in customer-defined Dextramers and/or collaboration with Immudex, please contact us on E-mail customer@immudex.com, or phone +45 20 437 665

If you need ordering information, please contact us on E-mail ordering@immudex.com, or phone +45 29 134 224