

Intracellular staining in combination with MHC Dextramer™ staining

MHC Dextramers may be used together with reagents for intracellular staining. For example MHC Dextramers can be used with IntraStain (Dako Code K2311) to allow simultaneous detection of MHC and intracellular antigens. Additional washing step prior to IntraStain Reagent A is essential for good results using MHC Dextramers together with the IntraStain procedure.

1. Transfer 50 µL (up to 10⁶ cells) of the cell suspension to be analyzed (whole blood or mononuclear cells) to a 12 x 75 mm polystyrene test tube.
2. Add 10 µL MHC Dextramer™ and mix with a vortex mixer. Incubate in the dark at room temperature for 10 minutes.
3. Add an appropriate volume of fluorochrome-conjugated antibody specific for the cell-surface antigen to be stained (e.g. anti-CD8, clone DK25). Incubate for 15 minutes in the dark at room temperature.
4. Add 2 mL PBS Working Solution and mix gently. Centrifuge at 300 x g for 5 minutes, then aspirate the supernatant.
5. Add 100 µL IntraStain Reagent A, Fixative. Mix gently with a vortex mixer to ensure that the cells are in suspension.
6. Incubate in the dark at room temperature for 15 minutes.
7. Add 2 mL PBS Working Solution and mix gently.
8. Centrifuge at 300 x g for 5 minutes, then aspirate the supernatant, leaving approximately 50 µL of fluid. Mix thoroughly to ensure that the cells are in suspension.
9. Add 100 µL IntraStain Reagent B, Permeabilization, to each test tube. Add an appropriate volume of fluorochrome-conjugated antibody specific for the intracellular antigen to be stained. Mix gently to ensure that the cells are in suspension.
10. Incubate in the dark at room temperature for 15 minutes.
11. Repeat steps 7 and 8.
12. Re-suspend the pellet in an appropriate fluid for flow cytometry analysis.
13. Analyze on a flow cytometer. If the samples are not analyzed immediately, they may be stored in the dark at 2-8 °C for up to 8 hours.

Examples

IntraStain Procedure in combination with MHC Dextramers

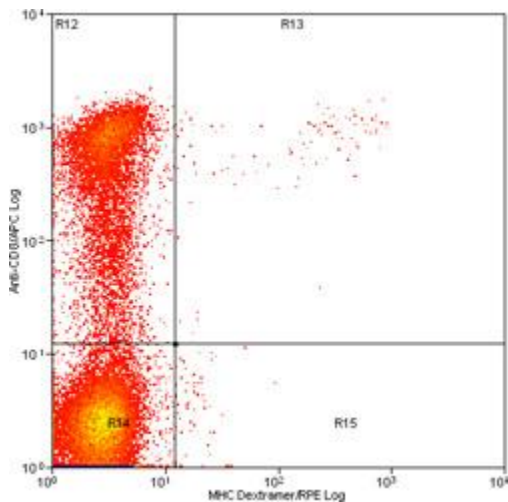


Figure A. Staining human peripheral blood lymphocytes with MHC/RPE Dextramer™ using IntraStain.

Human peripheral blood lymphocytes purified from a blood donor were stained with mouse anti-human CD3/PB antibody (Dako clone UCHT1, Code PB982) and mouse anti-human CD8/APC (clone DK25, Dako Code C7227) together with the specific Dextramer™ HLA-A*0201(NLVPMVATV)/RPE (Immudex Code WB2132-RPE). IntraStain (Dako Code K2311) was used for cell fixation and permeabilization, which allowed detection of intracellular antigens simultaneously with MHC detection. Samples were analyzed on a CyAn™ ADP Flow Cytometer. Live-gated CD3-positive lymphocytes are shown. Additional washing step prior to IntraStain Reagent A is essential for good results using MHC Dextramers together with the IntraStain procedure.

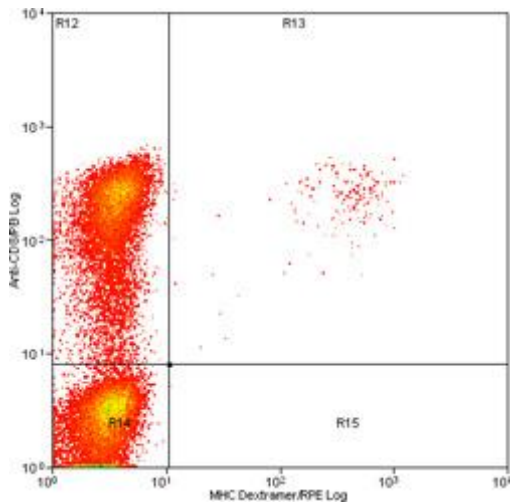


Figure B. Staining whole blood with MHC/APC Dextramer™ using IntraStain.

Human peripheral whole blood was stained with mouse anti-human CD3/APC antibody (clone UCHT1, Dako Code C7225) and mouse anti-human CD8/PB (clone DK25, Dako Code PB984) together with the specific Dextramer™ HLA-A*0201(NLVPMVATV)/RPE (Immudex Code WB2132-RPE). IntraStain (Dako Code K2311) was used for cell fixation and permeabilization, which allowed detection of intracellular antigens simultaneously with MHC detection. Samples were analyzed on a CyAn™ ADP Flow Cytometer. Live-gated CD3-positive lymphocytes are shown. Additional washing step prior to IntraStain Reagent A is essential for good results using MHC Dextramers together with the IntraStain procedure.

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