

# General Staining Procedure

As for all other protocols involving light-sensitive fluorochromes, MHC Dextramers staining should be carried out in the dark.

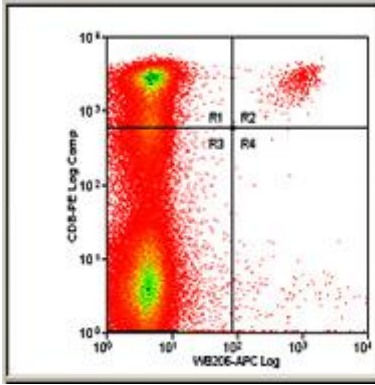
1. Transfer  $1-3 \times 10^6$  lymphoid cells (PBMC or spleenocytes) to a 12 x 75 mm polystyrene test tube. *The white blood cells may be isolated by Ficoll separation if high background levels appear.* Allocate only  $2-5 \times 10^5$  cells per tube when staining T-cell clones or cell lines due to the high frequency of antigen-specific T cells.
2. Add 2 mL 0.01 mol/L PBS containing 5% fetal calf serum and centrifuge at 300x g for 5 minutes. Remove supernatant and resuspend cells in remaining liquid.
3. Add 10  $\mu$ L of MHC Dextramer™ and mix gently with a vortex mixer. Incubate in the dark at room temperature for 10 minutes.
4. Add an optimally titrated amount of anti-CD8 antibody conjugated with a relevant fluorochrome (e.g. Dako clone DK25 for human lymphocytes or clone YTS169.4/KT15 for mouse lymphocytes). Incubate in the dark at 2-8 °C for 20 minutes.
5. Add 2 mL of 0.01 mol/L PBS containing 5% fetal calf serum and centrifuge at 300x g for 5 minutes, and remove supernatant.
6. 6. Optional wash: Add 2 mL of 0.01 mol/L PBS containing 5% fetal calf serum and centrifuge at 300x g for 5 minutes. This step will minimize background staining.
7. Re-suspend pellet in an appropriate fluid for flow cytometry, e.g. 0.4 mL PBS. 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.

## Note:

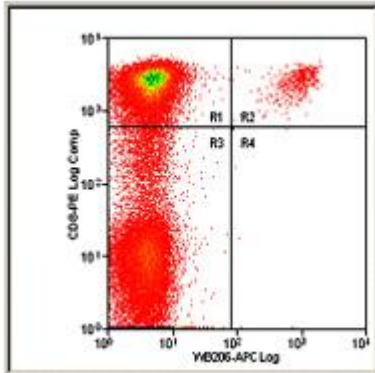
- Do not freeze.
- Some CD8 clones can inhibit MHC Dextramer™ staining. This can be avoided if the MHC Dextramers are stained first as listed above.
- Always keep MHC Dextramers stored at 2-8 °C in the dark - the brown plastic vials do not protect the reagent sufficiently against light.
- See Tips and Tricks for optimizing MHC Dextramer™ staining.

## Examples

### Staining Human Peripheral Blood Lymphocytes with MHC/APC Dextramers

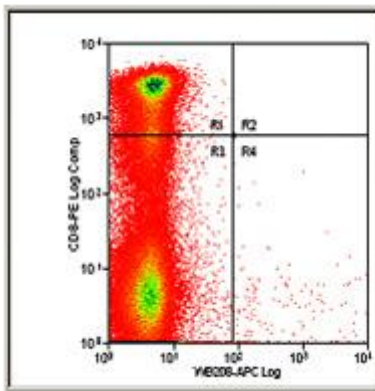


**Figure A.** Human peripheral blood lymphocytes stained with mouse anti-human CD8/RPE, clone DK25 (Dako Code R0806) and MHC Dextramer™ HLA-A\*0201 (NLVPMATV)/APC (Immudex Code WB2132-APC).



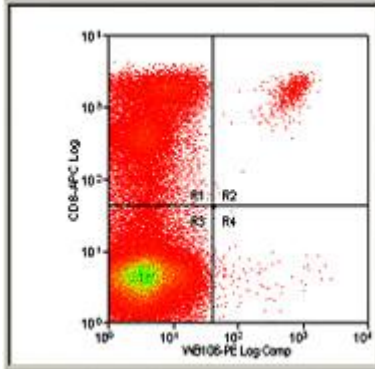
**Figure B.** Human peripheral blood lymphocytes stained with mouse anti-human CD8/RPE, clone DK25 (Dako Code R0806) and MHC Dextramer™ HLA-A\*0201 (NLVPMATV)/APC Immudex (Code WB2132-APC).

Cells were also gated for CD3-positive lymphocytes using anti-human CD3/FITC antibody, clone UCHT1 (Dako Code F0818).

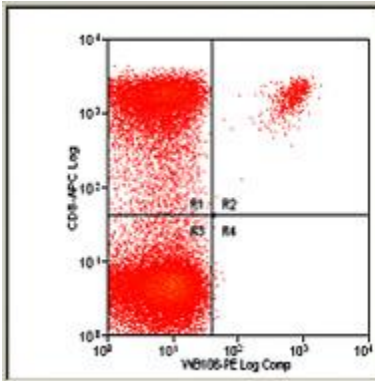


**Figure C.** Human peripheral blood lymphocytes stained with mouse anti-human CD8/RPE, clone DK25 (Dako Code R0806). MHC Dextramer™ HLA-A\*0201 (ILKEPVHGV)/APC (Code WB2139-APC) was used as a negative control.

## Staining Human Peripheral Blood Lymphocytes with MHC/RPE Dextramers

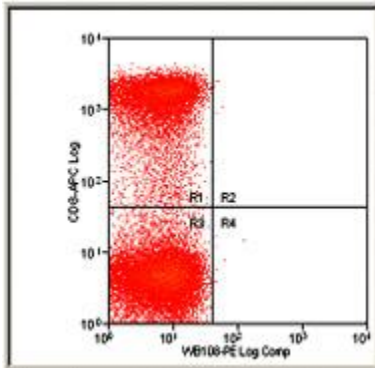


**Figure A.** Human peripheral blood lymphocytes stained with mouse anti-human CD8/APC, clone DK25 (Dako Code C7227), and MHC Dextramer™ HLA-A\*0201 (NLVPMATV)/RPE (Immudex Code WB2132-RPE).



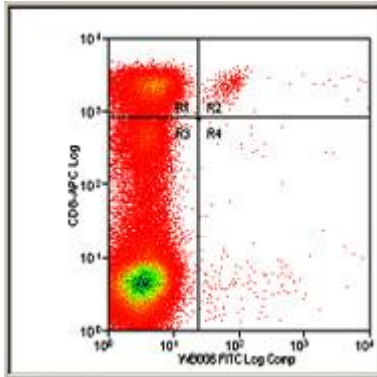
**Figure B.** Human peripheral blood lymphocytes stained with mouse anti-human CD8/APC, clone DK25 (Dako Code C7227), and MHC Dextramer™ HLA-A\*0201 (NLVPMATV)/RPE (Immudex Code WB2132-RPE).

Cells were also gated for CD3-positive lymphocytes using anti-human CD3/FITC antibody, clone UCHT1 (Dako Code F0818).

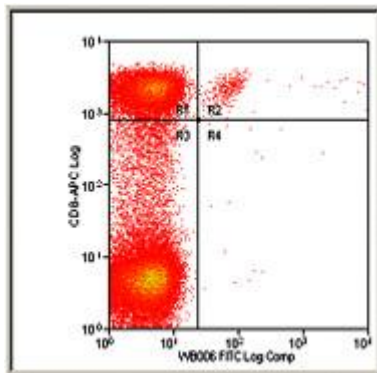


**Figure C.** Human peripheral blood lymphocytes stained with mouse anti-human CD8/APC, clone DK25 (Dako Code C7227). MHC Dextramer™ HLA-A\*0201 (ILKEPVHGV)/RPE (Immudex Code WB2139-RPE) was used as a negative control.

## Staining Human Peripheral Blood Lymphocytes with MHC/FITC Dextramers

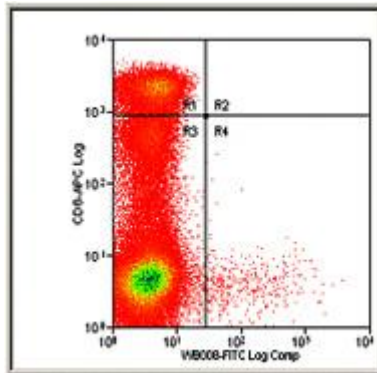


**Figure A.** Human peripheral blood lymphocytes stained with mouse anti-human CD8/APC, clone DK25 (Dako Code C7227) and MHC Dextramer™ HLA-A\*0201 (NLVPMATV)/FITC (Immudex Code WB2132-FITC).



**Figure B.** Human peripheral blood lymphocytes stained with mouse anti-human CD8/APC, clone DK25 (Dako Code C7227) and MHC Dextramer™ HLA-A\*0201 (NLVPMATV)/FITC (Immudex Code WB2132-FITC).

Cells were also gated for CD3-positive lymphocytes using anti-human CD3/RPE antibody, clone UCHT1 (Dako Code R0810).



**Figure C.** Human peripheral blood lymphocytes stained with mouse anti-human CD8/APC, clone DK25 (Dako Code C7227). MHC Dextramer™ HLA-A\*0201 (ILKEPVHGV)/FITC (Immudex Code WB2139-FITC) was used as a negative control.

### 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](mailto:customer@immudex.com), or phone +45 20 437 665

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