MHC Dextramer® Staining Protocol

Products

MHC I Dextramer® Cat. No. Wxxxxxx / Jxxxxxx [fluorochrome] [size]
MHC II Dextramer® Cat. No. Fxxxxxx [fluorochrome] [size]
CD1d Dextramer® Cat. No. XDxxxxx / YDxxxxx [fluorochrome] [size]
MR1 Dextramer® Cat. No. ZAxxxxx [fluorochrome] [size]
HLA-G Dextramer® Cat. No. USxxxxx [fluorochrome] [size]
HLA-E Dextramer® Cat. No. URxxxxx [fluorochrome] [size]
Collectively denominated as MHC Dextramer®.

Recommended use

Staining of antigen-specific T cells, NKT or MAIT cells using one or more fluorochrome-labelled MHC Dextramer® reagents in one sample.

Materials Provided

MHC Dextramer® with one of the following fluorochromes:
FITC [FI], PE, APC [AP], or without fluorochrome [NO].

Materials Required (not provided)

4 mL Falcon disposable 12 x 75-mm test tubes or equivalent
LoBind® Eppendorf tubes or equivalent
Stain and wash buffer: PBS, 1-5% FCS, pH 7.4
100 µM d-Biotin (e.g. Avidity, cat# BIO200) diluted in PBS, pH 7.4
10x PBS, pH 7.4
Antibodies identifying relevant cell surface markers:
For $CD8^+$ T, $CD4^+$ T and NKT cells (e.g., CD3, CD4 and CD8).
For MAIT cells (e.g. CD3, CD4, CD8 and CD161).
Optionally other desired antibodies and live-dead dye[A].
See the FAQ on immudex.com regarding recommended antibody clones.
The optimal choice of fluorochromes depends on the flow cytometer and experimental setup.

Procedure

1. Thaw and prepare PBMCs[B] and resuspend 1-3 x 10^6 PBMCs (for clonal cells, use 2-5 x 10^4 instead) in 50 µL stain and wash buffer.
2. To prepare a pool of multiple MHC Dextramer® reagents (*calculation example can be found in Appendix 1)*, mix the following reagents in an empty 1.5 mL LoBind® Eppendorf tube[C]:
    a. Add 0.2 µL of 100 µM d-Biotin[D] per Dextramer® reagent.
    b. Add 10 µL of each Dextramer® reagent.
    c. Add 0.6 µL of 10x PBS[D] per Dextramer® reagent.

NB: When staining with a single Dextramer® reagent, a and c can be omitted.

3. Vortex the Dextramer® pool briefly. The Dextramer® pool must be used directly after preparation and cannot be stored.
4. Centrifuge the pool at 10.000 x g for 1 min. to avoid transferring any potential precipitate.
5. Add the Dextramer® pool to the cell sample and vortex briefly.
6. Incubate in the dark at room temperature[E]:
    a. MHC I, CD1d, MR1, HLA-E[E], or HLA-G Dextramer® pool: 10 min. incubation[F].
    b. MHC II Dextramer® pool: 30 min. incubation[F].

   c. Dextramer® pool comprised of a. and b.: 30 min. incubation[F].
7. Add relevant antibodies in the volume/concentration according to manufacturer's instructions:
   a. If staining with MHC I Dextramer® reagents, use anti-CD3, anti-CD8[G], and optionally other phenotype markers.
   b. If staining with MHC II Dextramer® reagents, use anti-CD3, anti-CD4 and optionally other phenotype markers.
   c. If staining with CD1d Dextramer® reagents, use anti-CD3 anti-CD8[G] and anti-CD4 and optionally other phenotype markers.
   d. If staining with MR1 Dextramer® reagents, use anti-CD3 anti-CD8[G], anti-CD4, anti-CD161 and optionally other phenotype markers.
8. Incubate at room temperature in the dark for 20 min.
9. Wash cells by adding 2 mL stain and wash buffer. Centrifuge at 300 x g for 5 min. and remove the supernatant. Repeat washing for a total of 2 washes[H].
10. Resuspend the pellet in desired volume of stain and wash buffer suitable for your flow cytometer.
11. Proceed to analyze the samples on a flow cytometer or store at 2-8 °C in the dark. For optimal results, do not store the samples longer than 2 hours before acquisition. Alternatively, fixed cells[I] can be stored at 2-8 °C in the dark for up to 24 hours.

Procedural notes

A. Live-dead staining can be performed at the beginning or end of staining procedure according to manufacturer's instructions.
B. Dextramer® staining can be performed on any cell suspensions, cell lines, TILs, or whole blood, if the cells are non-fixed. For whole-blood samples, stain with Dextramer® reagents before Red Blood Cell (RBC) lysis or use non-fixable RBC lysing solution.
C. Always keep Dextramer® reagents stored at 2-8 °C in the dark – the plastic vial only partially protects the reagents against light.
D. d-biotin is required to avoid artefacts in the staining. 10x PBS will balance the salt concentration of the pool.
E. HLA-E Dextramer® should be kept at 2-8°C or on ice during general handling of the reagent, although the staining is performed at room temperature.
F. Incubation time may be increased when using a high number of reagents in pool staining and requires optimization.
G. Staining with antibodies against CD3 and CD8 has a negative impact on simultaneous or subsequent staining with MHC I Dextramer®. In most cases it is therefore highly recommended to stain with MHC I-, MR1- and CD1d-Dextramer® before staining with CD3 and CD8 antibodies. Simultaneous staining will reduce the Dextramer® staining intensity significantly.
H. Staining can be performed using 96-well microtiter plates. In that case after antibody incubation make 4 sequential washes using 200 µL stain and wash buffer per well. Centrifuge at 300 x g for 5 min. between each wash and remove supernatant.

I. Dextramer® stained cells can be fixed using 2% Methanol free formalin in PBS. Fixed samples may be washed and resuspended in stain and wash buffer prior to acquisition on a flow cytometer.

Technical support

For additional Tips & Tricks, FAQs and protocols, please visit https://www.immudex.com/resources/ or contact our support team at customer@immudex.com
Telephone: +45 3110 9292 (Denmark)

Analysis Guidelines

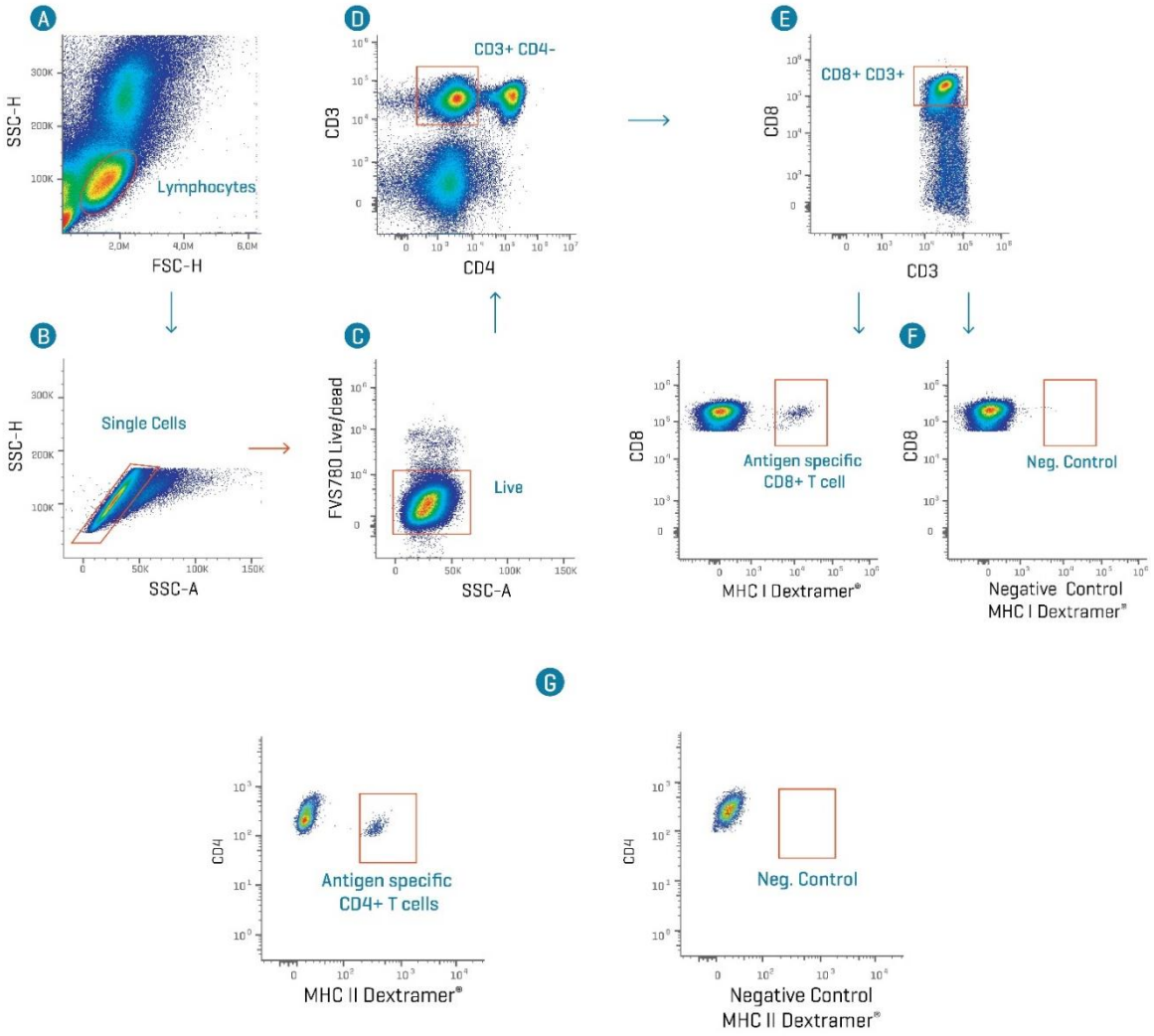

Fig. 1: Flow cytometry gating strategy using MHC I Dextramer® to identify antigen specific T-cells from samples of thawed hPBMCs. (A-F) gating of CD8⁺ antigen specific T cells. (**A**) Lymphocytes were identified based on the forward (FSC) - and side scatter (SSC) profiles. (**B**) Next, doublets were excluded by gating the single cells in a side scatter height (SSC-H) & side scatter area (SSC-A) profile plot. (**C**) Dead cells were excluded according to the live-dead stain (FVS780), and the live cells were gated for further characterization. (**D**) To exclude CD4⁺ T cells and Natural killer cells (NK) (positive for CD8 but not CD3), the CD3⁺/CD4- population was gated. (**E**) The CD3⁺/CD8⁺ T cells were then gated, and (**F**) subsequently, the antigen-specific population of cells were

determined by comparing the results of gating the MHC I Dextramer® labeled or MHC I Dextramer® Negative Control labeled cells. (**G**) Flow cytometry plots showing $CD4^+$ T helper cells labeled with MHC II Dextramer® or Negative Control MHC II Dextramer®.

Appendix 1
Calculation Examples

Preparation of pools of MHC Dextramer® reagents for staining 1 sample:

Examples	100 µM d-Biotin	Total MHC Dextramer® Reagents	10x PBS	Total Volume
Per MHC Dextramer® reagent	0.2 µL	10 µL per MHC Dextramer®	0.6 µL	10.8 µL
2 MHC Dextramer® reagents	0.4 µL	20 µL MHC Dextramer®	1.2 µL	21.6 µL
3 MHC Dextramer® reagents	0.6 µL	30 µL MHC Dextramer®	1.8 µL	32.4 µL
10 MHC Dextramer® reagents	2 µL	100 µL MHC Dextramer®	6 µL	108 µL

Preparation of pools of MHC Dextramer® reagents for staining 2 samples:
Note: When preparing a pool for more than 1 sample, we recommend preparing 20% overage of the pool, which is included in the examples below.

Examples	100 µM d-Biotin	Total MHC Dextramer® Reagents	10x PBS	Total Volume
Per MHC Dextramer® reagent	0.2 µL	12 µL per MHC Dextramer®	0.7 µL	12.9 µL
2 MHC Dextramer® reagents	0.5 µL	24 µL MHC Dextramer®	1.4 µL	25.9 µL
3 MHC Dextramer® reagents	0.7 µL	36 µL MHC Dextramer®	2.2 µL	38.9 µL
10 MHC Dextramer® reagents	2.4 µL	120 µL MHC Dextramer®	7.2 µL	129.6 µL