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:
BV421, 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
BD Horizon™ Brilliant Stain Buffer (cat# 563794; required only for stainings with MHC Dextramer® BV421)
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] by washing twice in 10 mL Stain and Wash buffer.
2. Resuspend 1-3 x 10^6 PBMCs as follows (for clonal cells, resuspend 2-5 x 10^4 cells):
    a. Resuspend cells in 50 µL Stain and Wash buffer (if using MHC Dextramer® PE, FITC or APC)
    b. Resuspend cells in 50 µL BD Horizon™ Brilliant Stain Buffer (if using MHC Dextramer® BV421)
3. 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.

4. Vortex the Dextramer® pool briefly. The Dextramer® pool must be used directly after preparation and cannot be stored.
5. Centrifuge the pool at 10.000 x g for 1 min. to avoid transferring any potential precipitate.
6. Add the Dextramer® pool to the cell sample and vortex briefly.
7. 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].
8. 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.
9. Incubate at room temperature in the dark for 20 min.
10. 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].
11. Resuspend the pellet in desired volume of stain and wash buffer suitable for your flow cytometer.
12. 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

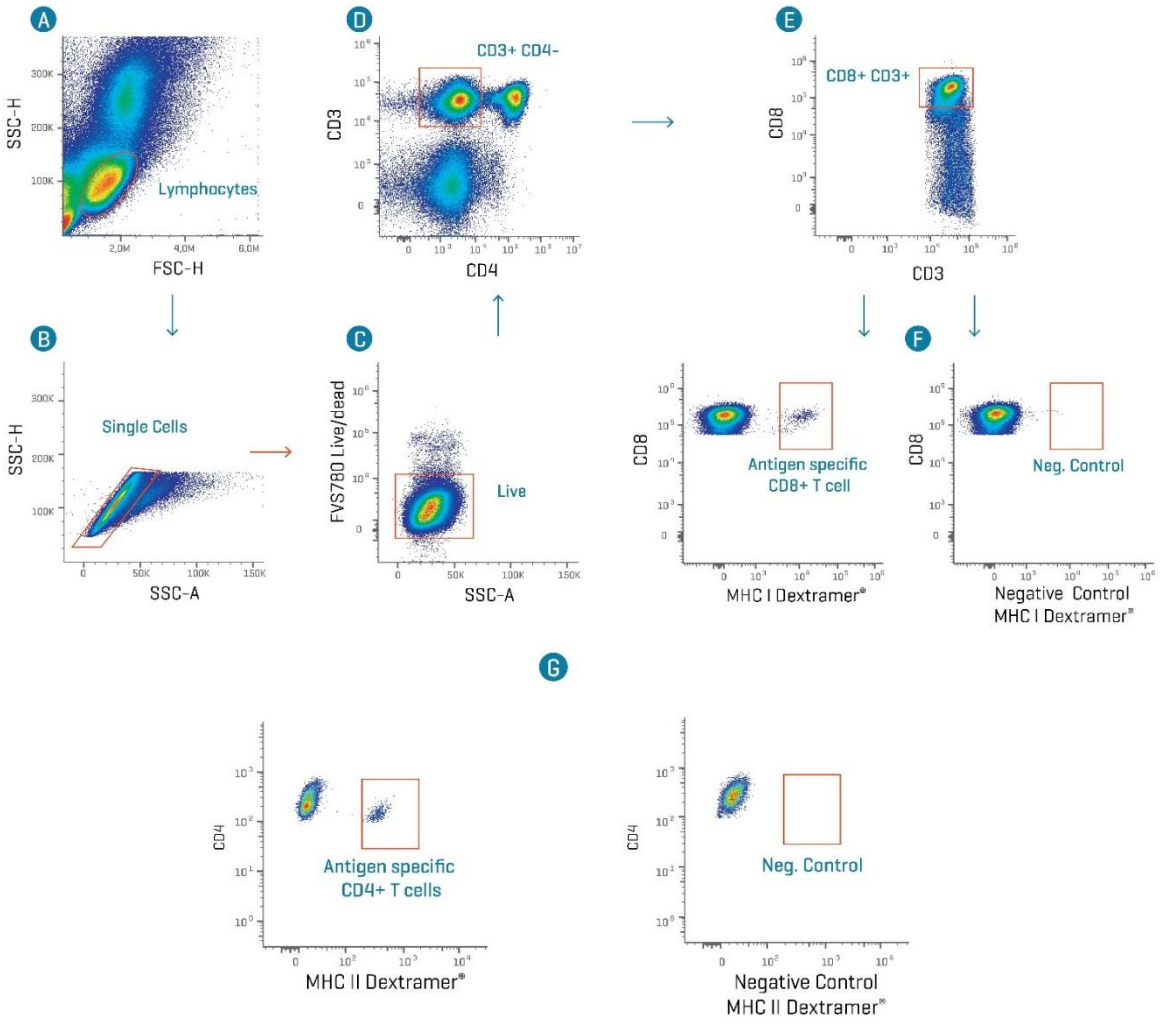

Fig. 1: Flow cytometry gating strategy using MHC I Dextramer® to identify antigen specific T-cells from samples of thawed hPBMCs. (A-F) gating of CD8⁺ antigen specific T cells. (**A**) Lymphocytes were identified based on the forward (FSC) - and side scatter (SSC) profiles. (**B**) Next, doublets were 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

Trademarks

BV421 is equivalent to Brilliant Violet™ 421, which is a trademark or registered trademark of Becton, Dickinson and Company or its affiliates, and is used under license. Powered by BD Innovation.

Label License

MHC Dextramer® with BV421 is provided under an intellectual property license from Becton, Dickinson and Company. The purchase of this product conveys to the buyer the non-transferable right to use the purchased product and components of the product only in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The sale of this product is expressly conditioned on the buyer not using the product or its components, or any materials made using the product or its components, in activity to generate revenue including (a) in manufacturing other than limited to quality control; (b) for therapeutic, diagnostic or prophylactic purposes; or (c) for resale, regardless of whether they are resold for use in research. For information on purchasing a license to this product for purposes other than research, contact Becton, Dickinson and Company, 10975 Torreyana Road, San Diego, California 92121 USA or bdblicensing@bd.com.