MHC Dextramer® Staining Protocol – Multiple Reagents

Intended use

Staining of antigen-specific T cells using multiple fluorochrome-labelled MHC Dextramer® reagents in one sample.

Materials Provided

MHC Dextramer® PE, APC or FITC

Materials Required (not provided)

Stain and wash buffer: PBS, 1-5% FCS, pH 7.4
100 µM d-Biotin in PBS, pH 7.4
10x PBS, pH 7.4
Antibodies identifying relevant surface markers (e.g., CD3, CD4, CD8 and optionally other desired antibodies). The optimal choice of antibodies and fluorochromes depends on the flow cytometer and experimental setup.

Procedure

1. Thaw and prepare PBMC sample and resuspend 1-3 x 10^6 PBMC 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 a 1.5 mL tube:
    a. Add 1 µL of 10x PBS per MHC Dextramer® reagent into an empty tube.
    b. Add 0.2 µL of 100 µM d-Biotin per MHC Dextramer® reagent.
    c. Add 10 µL of each MHC Dextramer® reagent.
3. Vortex the MHC Dextramer® pool briefly. The MHC Dextramer® pool must be used directly after preparation and cannot be stored.
4. Centrifuge the pool at 10,000 x g for 1 min.
5. Add the MHC Dextramer® pool to the cell sample and vortex briefly. *Make sure not to transfer any precipitate from the bottom of the tube.*
6. Incubate at room temperature in the dark, for 10 min. *MHC II Dextramer® reagents require longer incubation. See Procedural notes.*
7. Add relevant antibodies in the volume/concentration recommended by the provider:
    a. If staining with MHC I Dextramer® reagents, use anti-CD8, anti-CD3, and optionally other phenotype markers.
    b. If staining with MHC II Dextramer® reagents, use anti-CD4, anti-CD3, 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.
10. Resuspend the pellet in 300 µL or desired volume 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, samples can be fixated. For fixated samples, see Procedural notes.*

Procedural notes

1. If you intend to stain with a single MHC Dextramer® reagent, follow the protocol "MHC Dextramer® Staining Protocol – Single Reagent". (https://www.immudex.com/resources/protocols/)
2. MHC Dextramer® staining can be performed on whole blood.
3. Protocol step 6: When staining with MHC II Dextramer® reagents, a mixed MHC I and II Dextramer® pool or identifying low affinity targets, stain for 30 min.
4. Protocol step 7: 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.
5. Protocol step 11: MHC Dextramer® stained cells can be fixated using 2% formaldehyde in PBS. Fixated samples can be stored in the dark for up to 24 hours before acquisition on a flow cytometer. Fixated samples should be washed and resuspended in stain and wash buffer prior to acquisition on a flow cytometer.
6. Always keep MHC Dextramer® reagents stored at 2-8°C in the dark – the plastic vial only partially protects the reagents against light.

Storage

Store in the dark at 2-8°C.

Precautions

Contains sodium azide (NaN_3), a chemical highly toxic in pure form. At product concentrations, though not classified as hazardous, sodium azide may react with lead and copper plumbing to form highly explosive build-ups of metal azides. Upon disposal, flush with large volumes of water to prevent metal azide build-up in plumbing. As with any product derived from biological sources, proper handling procedures should be used.
For professional users.

Symbols

See www.immudex.com/symbols for explanation of symbols.

Technical support

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Appendix 1

Calculation Example Preparation of pools of MHC Dextramer® reagents:

Examples	10x PBS	100 µM d-Biotin	MHC Dextramer® Reagents	Total Volume
3 MHC Dextramer® reagents	3 µL	0.6 µL	10 µL per reagent	33.6 µL
10 MHC Dextramer® reagents	10 µL	2 µL	10 µL per reagent	112 µL