Staining Protocol - dCODE™ Dextramer® (10x compatible)

For research use only (RUO). Not for use in diagnostic procedures.

Purpose

Staining of antigen-specific T cells in PBMC sample using dCODE™ Dextramer® (10x compatible) reagents

Required materials

Stain and wash buffer: PBS, pH 7.4, containing 1-5% Serum
d-Biotin 100µM in PBS pH 7.4 or similar buffer
dCODE™ Dextramer® (10x compatible) reagents
Optional: antibodies identifying relevant cell surface markers (e.g. CD3, CD4, CD8)

Staining procedure

Staining with dCODE™ Dextramer® must be kept shielded from light.

1. Prepare PBMC sample and resuspend cells in Stain and wash buffer. 2 µl dCODE™ Dextramer® reagent is enough to stain 1-3 x 10^6 PBMC in a volume of 50 – 100 µl.
2. Preparation of dCODE™ Dextramer® reagent pool:
    a. In an empty tube, add 0.2 µl 100µM d-Biotin per dCODE™ Dextramer® specificity
    b. Add 2 µl of each dCODE™ Dextramer® specificity, mix
3. Add the pool of dCODE™ Dextramer® reagents to the cell sample and mix thoroughly.
4. Incubate at room temperature for 10 min.
5. Add relevant antibodies in volume and concentration recommended by provider. Incubate for 20 min. If no antibodies are added continue incubation with dCODE™ Dextramer® reagent pool for additional 20 min.
6. Washing:
    a. If staining in 4 ml tubes, add 2 ml Stain and wash buffer. Centrifuge at 300 x g for 5 min. and remove the supernatant. Repeat washing with additional 2ml stain and wash buffer.
    b. If staining in 96-well microtiter plates, 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.
7. Resuspend cells in adequate volume (as recommended by 10x Genomics) of either of the buffers:
    a. PBS (Calcium, Magnesium free) + 0.04% W/V BSA, or
    b. PBS, Dulbecco's Phosphate-Buffered Saline (DPBS), Hank's Balanced Salt Solution (HBSS), Eagle's Minimum Essential Medium (EMEM), or Dulbecco's Modified Eagle Medium (DMEM) + 10% FBS
8. Proceed to 10x Genomics Feature Barcode Protocol:
    - CG000186_ChromiumSingleCellV(D)J_ReagentKit_FeatureBarcodingtechnology
    - CG000126_Cell_counting_flowchart
    - Single Cell Protocols - Cell Preparation Guide

Notes:

Cell viability is crucial for successful Single Cell Immune Profiling assay. We recommend determining the cell concentration and viability, of your cell sample after staining and before introducing the cells into the Chromium chip. Cell viability should be >70%.

Antigen-specific T Cells are of low frequency in peripheral blood. Enough cells must be stained and analysed for detecting rare cell populations. Alternative, a pre-enrichment step may be introduced to enrich the dCODE™ Dextramer® positive cells, before applied to the Chromium Chip.

Read more about Single Cell Immune Profiling, and find relevant support tools at

- www.10xgenomics.com
- Single Cell Immune Profiling Support
- Immune Profiling Feature Barcoding