Staining Protocol - dCODE Dextramer® (10x)

Recommended use

Single-cell multi-omics analysis of antigen-specific T, NKT, or MAIT cells in cell samples, using the 10x Chromium Single Cell Gene Expression Platform

Products

dCODE Dextramer® (10x), cat# (U)WBxxxxxdXG / FBxxxxxdXG
CD1d dCODE Dextramer® (10x), cat# XDxxxxxdXG / YDxxxxxdXG
MR1 dCODE Dextramer® (10x), ZAxxxxxdXG
Collectively denominated as dCODE® (10x)

Materials Provided

dCODE Dextramer® (10x)
and/or CD1d dCODE Dextramer® (10x)
and/or MR1 dCODE Dextramer® (10x)

Materials Required (not provided)

Stain buffer: PBS, pH 7.4 containing 1-5% Serum and 0,1 g/L Herring sperm DNA
Wash buffer: PBS, pH 7.4 containing 1-5% Serum
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.

Procedure

Staining with dCODE® (10x) must be kept shielded from light.

1. Prepare PBMC sample and resuspend cells in stain buffer. 2 µL dCODE® (10x) reagent is enough to stain 1-3 x 106 PBMC in a volume of 50 – 100 µL.
2. Preparation of dCODE® (10x) reagent pool:
   a. In an empty tube, add 0.2 µL 100µM d-Biotin per dCODE® (10x) specificity
   b. Add 2 µL of each dCODE® (10x) specificity, mix
3. Add the pool of dCODE® (10x) reagents to the cell sample and mix thoroughly
4. Incubate in the dark at room temperature:
   a. MHC I, MR1 or CD1d dCODE Dextramer® (10x) pool: 10 min. incubation[A].
   b. MHC II dCODE Dextramer® (10x) pool: 30 min. incubation[A].
   c. dCODE® (10x) pool comprized of a. and b: 30 min. incubation[A].
5. Add relevant antibodies in volume and concentration recommended by provider. Incubate for 20 min.
   NB: 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 wash buffer. Centrifuge at 300 x g for 5 min. and remove the supernatant. Repeat washing with additional 2 mL wash buffer.

b. If staining in 96-well microtiter plates, make 4 sequential washes using 200 µL 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:
   - CG000208 Chromium Next GEM Single Cell V(D)J Reagent Kit Feature Barcoding technology
   - CG000126_Cell_counting_flowchart
   - Single Cell Protocols - Cell Preparation Guide

Procedural Notes

A. Incubation time may be increased when using a high number of reagents in pool staining and requires optimization.

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® (10x) 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

Technical Support

For additional Tips & Tricks, FAQs and protocols, please visit https://www.immudex.com/resources/

Immudex® is the sole manufacturer and provider of dCODE Dextramer® (10x) reagents, and support related to these products is through Immudex.

E-mail Immudex customer support: customer@immudex.com
Telephone: +45 3110 9292 (Denmark)