DEMONSTRATED PROTOCOL

Cell Labeling with Dextramers for Single Cell RNA Sequencing Protocols

with Feature Barcoding technology

Overview

Multimeric MHC peptide complexes, such as Dextramers, bind to T-cell receptors (TCRs) with high affinity, which can enable detection of TCR antigen specificity. This protocol provides guidance for labeling cells with dCODE Dextramer (Dextramer MHC-Feature Barcode oligonucleotide conjugate), along with guidance for enriching Dextramer$^+$ T cells by Fluorescence Activated Cell Sorting (FACS). These Dextramer labeled cells can be used for generating Chromium Single Cell libraries as described in the User Guide for Chromium Immune Profiling Solution with Feature Barcoding technology (CG000186).

Additional Guidance

Consult Demonstrated Protocol Cell Preparation Guide (Document CG00053) for Tips & Best Practices on handling cells and Technical Note Guidelines on Accurate Target Cell Counts (Document CG000091) for determining accurate cell counts.

Cells carry potentially hazardous pathogens. Follow material supplier recommendations and local laboratory procedures and regulations for the safe handling, storage and disposal of biological materials.

Preparation – Buffers

Buffers Maintain at 4°C	Composition
Biotin Stock Solution	100 µM D-Biotin in PBS
Dextran Sulfate Solution	1% w/v (10 mg/ml) Dextran Sulfate Sodium Salt in Nuclease-free Water
Resuspension Buffer	PBS + 0.04% BSA
PBS + 2% FBS (maintain at 4°C) PBS + 10% FBS (maintain at 4°C)	

Specific Reagents & Consumables

Vendor	Item	Part Number
Immudex	dCODE Dextramers	-
Millipore Sigma	Phosphate-Buffered Saline (PBS) with 10% Bovine Albumin (alternative to Thermo Fisher product)	SRE0036
Corning	Phosphate-Buffered Saline, 1X without Calcium and Magnesium	21-040-CV
Thermo Fisher Scientific	UltraPure Bovine Serum Albumin (BSA, 50 mg/ml)	AM2616
	Countess II FL Automated Cell Counter	AMAQAF1000
	Countess II FL Automated Cell Counting Chamber Slides	C10228
	Trypan Blue Stain (0.4%)	T10282
BioLegend	Human TruStain FcX (Fc Receptor Blocking Solution)	422301
	TotalSeq-C Antibody-Oligonucleotide Conjugate	-
	Antibodies (Fluorophore) If enriching Dextramer$^+$ cells through FACS	-
VWR	Fetal Bovine Serum (FBS)	97068-085
MP Biomedicals	D-Biotin (>98% purity)	194634
	Dextran Sulfate Sodium Salt	101516
Fisher Scientific	Dextran Sulfate Sodium Salt (alternative to MP Biomedicals product)	AC441490050

Protocol Overview

1. Label Cells

Prepare following as described in the Cell Labeling Protocol:

- Dextramer Mix
- FACS Antibody Pool (if performing FACS enrichment of Dextramer$^+$ T cells)
- Antibody Mix (if simultaneously labeling with antibody-oligonucleotide conjugates)

Centrifuge cells for labeling (**350 rcf, 5 min**)
Remove supernatant

↓

Resuspend pellet
(in 100 µl PBS + 2% FBS)

↓

Add Human TruStain FcX (**5 µl**)
Dextran Sulfate Solution (**2 µl**)

↓

Add prepared Dextramer Mix

↓

Incubate at **4°C (10 min)**

↓

Add prepared FACS Antibody Pool
(If simultaneously labeling with antibody-oligonucleotide conjugates, add prepared Antibody Mix)
Gently pipette mix

↓

Incubate at **4°C (30 min)**

2. Wash Cells

See Appendix for buffer volumes when working with 15-ml tubes

Repeat 2x
(Total 3 washes)

Add PBS + 2% FBS to cells (**1.5 ml**)

↓

Centrifuge (**350 rcf, 5 min**)
Remove supernatant

↓

Enriching Dextramer$^+$ T cells?

No → If not performing FACS enrichment of Dextramer$^+$ T cells, resuspend cells in Resuspension Buffer and proceed to Chromium Single Cell Immune Profiling Solutions User Guide with Feature Barcoding technology (CG000186)

Yes

↓

Resuspend pellet
(in PBS + 2% FBS, includes a dead cell marker)
Gently pipette mix

↓

Proceed to FACS

↓

Proceed to Chromium Single Cell Immune Profiling Solutions User Guide with Feature Barcoding technology (CG000186)

Cell Labeling Protocol

This cell labeling protocol was optimized using dCODE Dextramers from Immudex and TotalSeq-C antibody-oligonucleotide conjugates from BioLegend. See Appendix for an illustrative overview of dCODE Dextramer capture by 10x Gel Bead primers. The labeled cells were further enriched for Dextramer$^+$ T cells by FACS sorting to enable identification of low frequency TCR:antigen binding events.

1. Label Cells

Prepare Dextramer Mix:

Add **0.2 µl** Biotin Stock for each specific Dextramer to a 1.5-ml microcentrifuge tube. Add **2 µl** of each Dextramer per reaction. Gently pipette mix and maintain at **4°C**. Avoid light exposure.

Prepare FACS Antibody Pool:

Add appropriate/manufacturer's recommended amount of fluorophore antibodies to a 1.5-ml microcentrifuge tube on ice. Gently pipette mix and maintain at **4°C**.
See Appendix for an example of the antibodies that can be used.

Prepare Antibody Mix
If simultaneously labeling with antibody-oligonucleotide conjugates:

Add appropriate/manufacturer's recommended amount of antibody-oligonucleotide conjugates to a 1.5-ml microcentrifuge tube. To avoid antibody aggregates, centrifuge the mix at **14,000 rcf** for **10 min** at **room temperature**. Transfer the supernatant (containing the Antibody Mix) to a new tube and maintain at **4°C**.

This protocol was demonstrated using 3-20 x 10^6 cells.

a. Centrifuge cells at **350 rcf** for **5 min** at **4°C.** Use of swinging-bucket rotor is recommended for higher cell recovery.

b. Remove the supernatant without disrupting the pellet.

c. Resuspend cell pellet in PBS + 2% FBS **(100 µl** for 3-10 x 10^6 cells).

d. Add **5 µl** (1:20 dilution) Human TruStain FcX.

e. Add **2 µl** (1:50 dilution) Dextran Sulfate Solution. Gently pipette mix.

f. Add prepared Dextramer Mix. DO NOT add FACS Antibody Pool and/or Antibody Mix at this step.

g. Incubate for **10 min** at **4°C.**

h. Add FACS Antibody Pool. If also labeling with antibody-oligonucleotide conjugates, add Antibody Mix supernatant at this step.

i. Incubate for **30 min** at **4°C.**

2. Wash Cells

To eliminate non-specific binding, wash steps may be performed either in 1.5-ml microcentrifuge tubes or 15-ml tubes using indicated buffer volumes. If concerned about dislodging the pellet during supernatant removal, perform wash steps in 15-ml tubes (see Appendix). Non-specific binding contributes to increased background reads during sequencing.

Optimization of centrifugation speed/time may be needed based on cell type.

a. Wash by adding **1.5 ml** PBS + 2% FBS to the cells from step 1i.

b. Centrifuge at **350 rcf** for **5 min** at **4°C** . Larger or fragile cell types may require slower centrifugation speeds.

c. Remove the supernatant without touching the bottom of the tube to avoid dislodging the pellet.

Leaving behind excess supernatant may cause non-specific binding, which may result in increased background reads during sequencing.

Pellet (may not be visible)

d. Using a **wide-bore** pipette tip, resuspend the cell pellet in **1.5 ml** PBS + 2% FBS.

e. Centrifuge at **350 rcf** for **5 min** at **4°C**.

f. Remove the supernatant without touching the bottom of the tube to avoid dislodging the pellet.

g. Repeat d - f for a total of three washes.

h. OPTIONAL. For enrichment of Dextramer$^+$ T cells by FACS: Based on starting concentration and assuming ~50% cell loss, add an appropriate volume PBS + 2% FBS (including a dead cell marker) to obtain a cell concentration of 5-10 x 10^6 cells/ml and proceed to FACS (see FACS Guidance). After FACS, proceed **immediately** to Chromium Single Cell Immune Profiling Solutions User Guide with Feature Barcoding technology (CG000186).

Cell loss depends on the starting cell numbers. Lower the starting cell number, higher would be the cell loss.

i. If not performing FACS:
Based on starting concentration and assuming ~50% cell loss, add an appropriate volume Resuspension Buffer to obtain a concentration of 700-1,200 cells/µl. Determine cell concentration and viability using a Countess II Automated Cell Counter. Proceed **immediately** to Chromium Single Cell Immune Profiling Solutions User Guide with Feature Barcoding technology (CG000186).

10x GENOMICS

Appendix

FACS Guidance

FACS sorting of Dextramer$^+$ T cells prior to the analysis of antigen-specific cell populations enables enrichment and identification of low frequency TCR:antigen binding events.

FACS Cell Collection

It is recommended to collect FACS enriched cells in PBS + 10% FBS to maintain cell viability. Cells should be collected either in 10-20 µl volume in the collection tube/plate (96-well plate) or 50 µl volume in a 1.5-ml tube.

The sort stream should be adjusted so that the cell-droplet falls into the collection buffer. Sorted cells must be counted and viability measured before proceeding to the 10x Genomics Single Cell protocols. If necessary, the collected cells may be concentrated by centrifugation at 350 rcf and removing the supernatant.

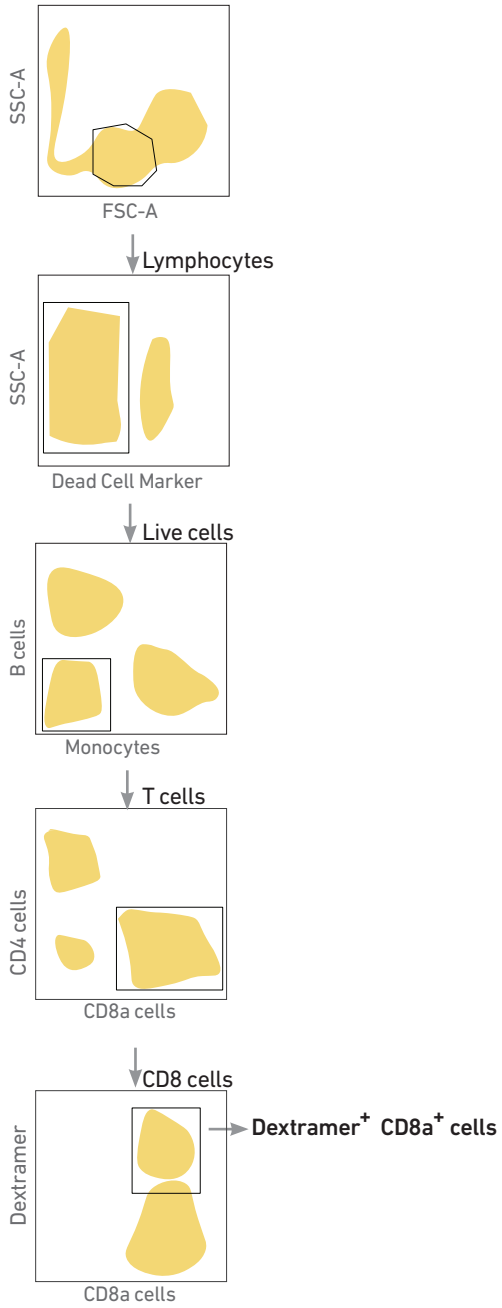
Cell loss during FACS is common. Optimize the protocol steps accordingly.

Example FACS Antibody Pool & Gating Strategy

This protocol was optimized using following fluorophore antibodies for FACS:

- CD14, CD15 and CD16 to exclude monocytes
- CD19 to exclude B cells
- CD4 to exclude CD4$^+$ T cells
- 7AAD to exclude dead cells
- CD8a to include CD8a$^+$ T cells

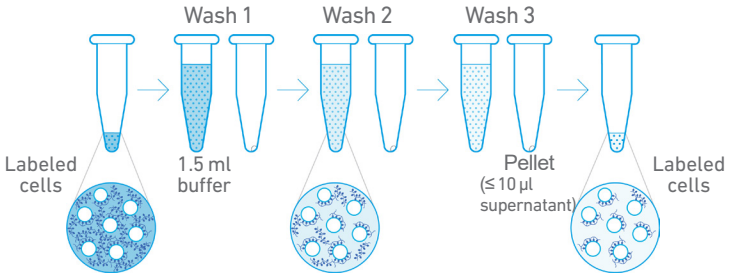
Figure 1. Example gating strategy using above mentioned antibodies



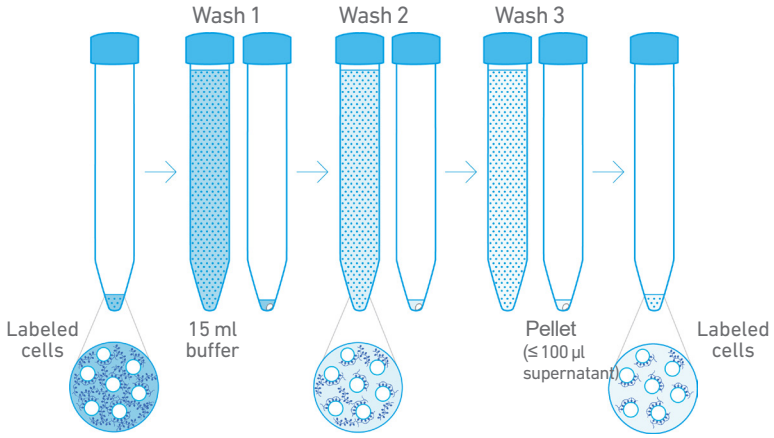
Illustrative Overview of Wash Steps

An illustrative overview of wash steps for Dextramer labeled cells is shown below:

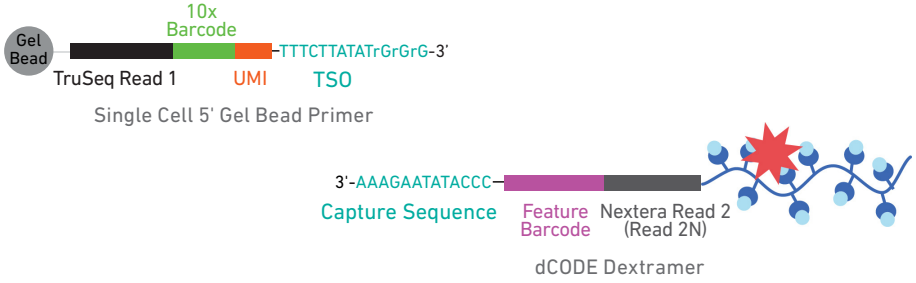
Wash Steps in 1.5-ml Tubes



Wash Steps in 15-ml Tubes



Illustrative Overview of dCODE Dextramer Capture



References

- Chromium Single Cell V(D)J Reagent Kits User Guide with Feature Barcoding technology for Cell Surface Protein (Document CG000186)

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