

## DEMONSTRATED PROTOCOL

# Cell Labeling with Dextramer Reagents for Single Cell RNA Sequencing Protocols

with Feature Barcoding technology

## Overview

Multimeric MHC peptide complexes, such as Dextramer reagents, 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® reagents (Dextramer MHC-Feature Barcode oligonucleotide conjugate), along with guidance for enriching Dextramer reagent<sup>+</sup> T cells by Fluorescence Activated Cell Sorting (FACS). These Dextramer reagents labeled cells can be used for generating Chromium Single Cell libraries as described in the User Guide for Chromium Single Cell Immune Profiling Solution with Feature Barcoding technology (CG000186 and CG000208).

## 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
Resuspension Buffer	PBS + 0.04% BSA
Dextran Sulfate Solution Only if adding Dextran Sulfate in step 1	1% w/v (10 mg/ml) Dextran Sulfate Sodium Salt in Nuclease-free Water
	PBS + 2% FBS (maintain at 4°C)

## Specific Reagents & Consumables

Vendor	Item	Part Number
Immudex	dCODE™ Dextramer® Reagents	-
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 reagent <sup>+</sup> cells through FACS	-
VWR	Fetal Bovine Serum (FBS)	97068-085
MP Biomedicals	D-Biotin (>98% purity)	194634
	Dextran Sulfate Sodium Salt (Optional)	101516
Fisher Scientific	Dextran Sulfate Sodium Salt (Optional; alternative to MP Biomedicals product)	AC441490050

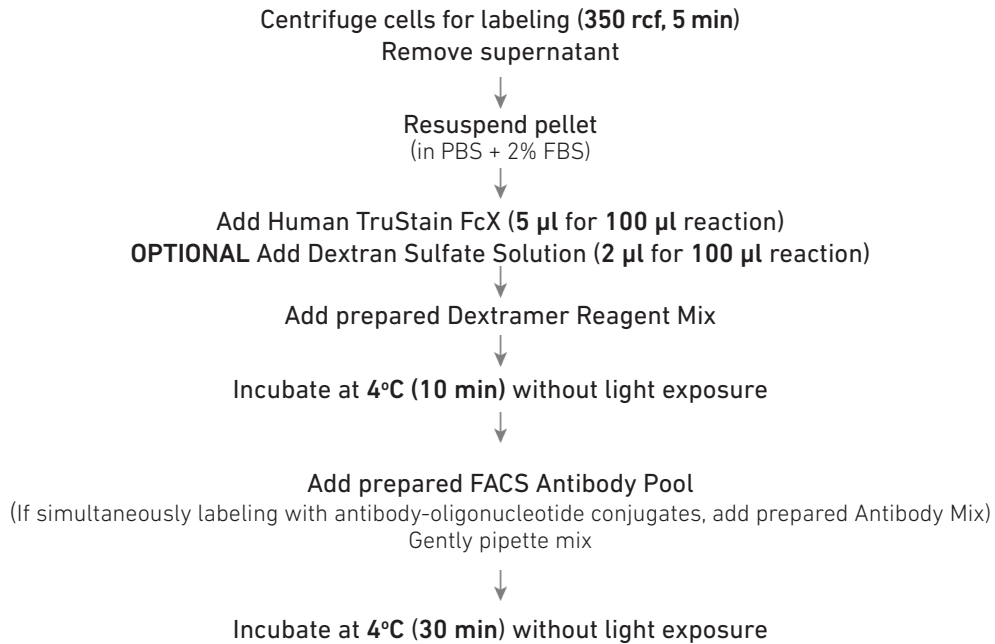
\*Choose different clones than antibody-oligonucleotide conjugates

## Protocol Overview

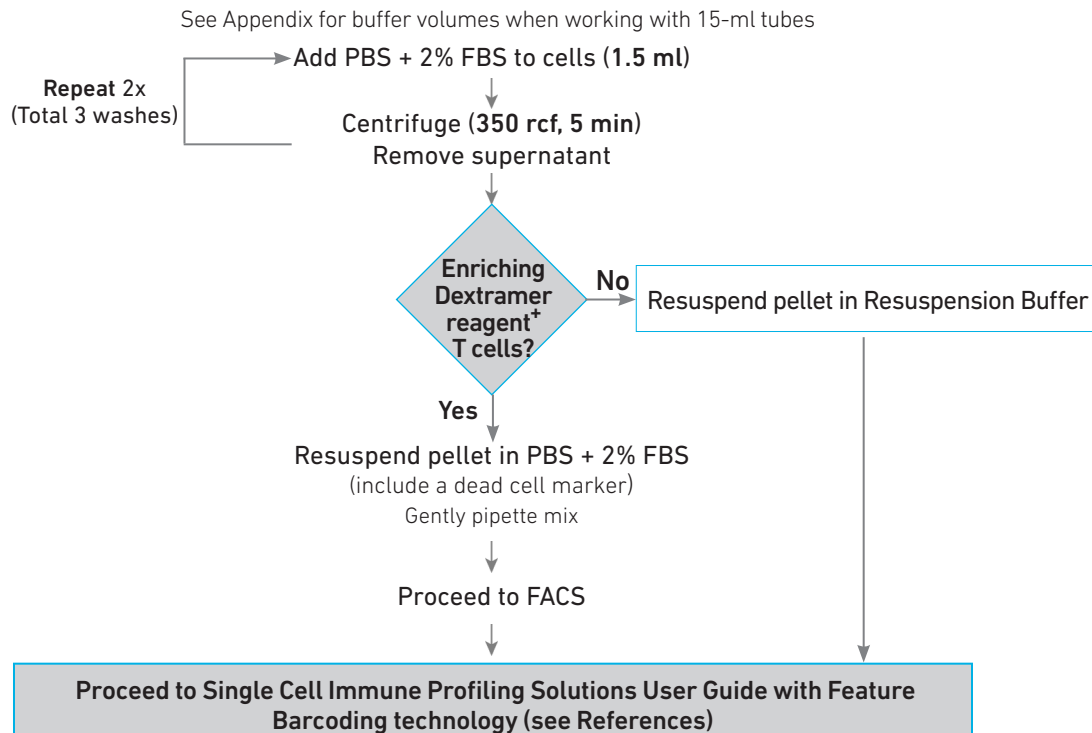
### 1. Label Cells

Prepare following as described in the Cell Labeling Protocol:

- Dextramer Reagent Mix
- FACS Antibody Pool (for performing FACS enrichment of Dextramer reagent<sup>+</sup> T cells)
- Antibody Mix (if simultaneously labeling with antibody-oligonucleotide conjugates)



### 2. Wash Cells



## Cell Labeling Protocol

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



Use distinct antibody clones for FACS and cell surface protein labeling. Optimize working concentration of each of the antibodies used.

### 1. Label Cells

#### Prepare Dextramer Reagent Mix:

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

#### Prepare FACS Antibody Pool:

Add appropriate/manufacture'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/manufacture'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 3-10 x 10<sup>6</sup> cells. Total reaction volume for labeling is **100 µl**.

- Centrifuge cells at **350 rcf** for **5 min** at **4°C**. Use of swinging-bucket rotor is recommended for higher cell recovery.
- Remove the supernatant without disrupting the pellet.
- Resuspend pellet in an appropriate volume PBS + 2% FBS. The resuspension volume depends upon the volumes of Dextramer Reagent Mix, FACS Antibody Pool, and Antibody Mix.
- Add **5 µl** (1:20 dilution of **100 µl** reaction) Human TruStain FcX. Gently pipette mix.

#### OPTIONAL

To reduce non-specific background, Dextran Sulfate Solution (1:50 dilution of **100 µl** reaction) may be added at this step. The use of Dextran Sulfate can be omitted, if concerned about its affect on cell viability.

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

- Incubate for **10 min** at **4°C** without light exposure.
- Add FACS Antibody Pool. If also labeling with antibody-oligonucleotide conjugates, add Antibody Mix supernatant at this step. If needed, add PBS + 2% FBS for a total volume of **100 µl**.
- Incubate for **30 min** at **4°C** without light exposure.

### 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.

- Wash by adding **1.5 ml** PBS + 2% FBS to the cells from step 1h.
- Centrifuge at **350 rcf** for **5 min** at **4°C**. Larger or fragile cell types may require slower centrifugation speeds.
- 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)

- Using a **wide-bore** pipette tip, resuspend the cell pellet in **1.5 ml** PBS + 2% FBS.
- Centrifuge at **350 rcf** for **5 min** at **4°C**.
- Remove the supernatant without touching the bottom of the tube to avoid dislodging the pellet.
- Repeat d - f** for a total of three washes.
- OPTIONAL For enrichment of Dextramer reagent<sup>+</sup> 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<sup>6</sup> 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 (see References).
- 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 and proceed **immediately** to Chromium Single Cell Immune Profiling Solutions User Guide with Feature Barcoding technology (see References).

## Appendix

### FACS Guidance

Enrich Dextramer reagent<sup>+</sup> T cells prior to the analysis of antigen-specific cell populations to enable enrichment and identification of low frequency TCR:antigen binding events.

#### FACS Cell Collection

It is recommended to collect FACS enriched cells in up to 100% FBS to maintain cell viability. Cells should be collected either in 20  $\mu$ l volume in the collection tube/plate (96-well plate) or 200  $\mu$ l 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.



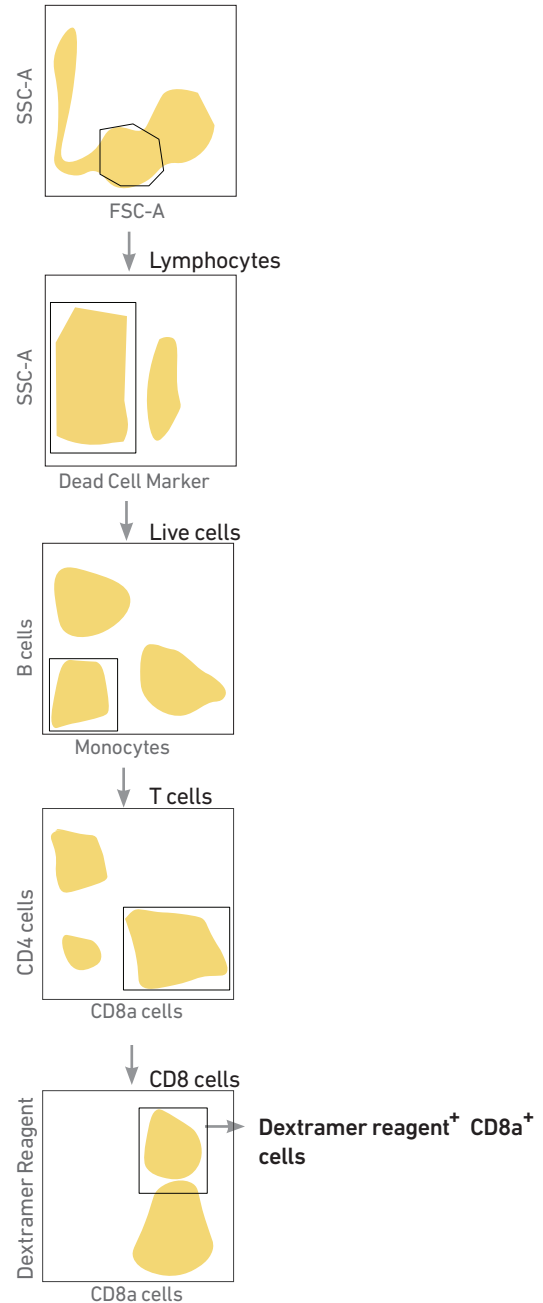
Once sorting is complete, proceed **immediately** to Chromium Single Cell Immune Profiling Solutions User Guide with Feature Barcoding technology (see References).

#### 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<sup>+</sup> T cells
- 7AAD to exclude dead cells
- CD8a to include CD8a<sup>+</sup> T cells

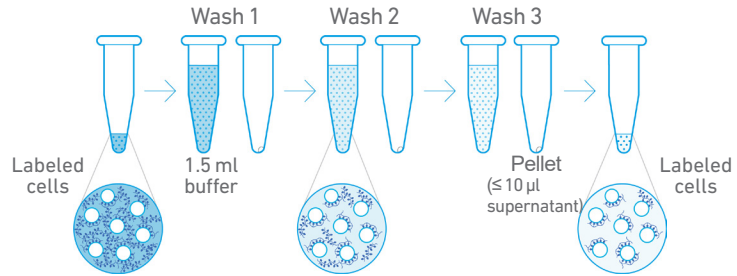
**Figure 1.** Example gating strategy using above mentioned antibodies



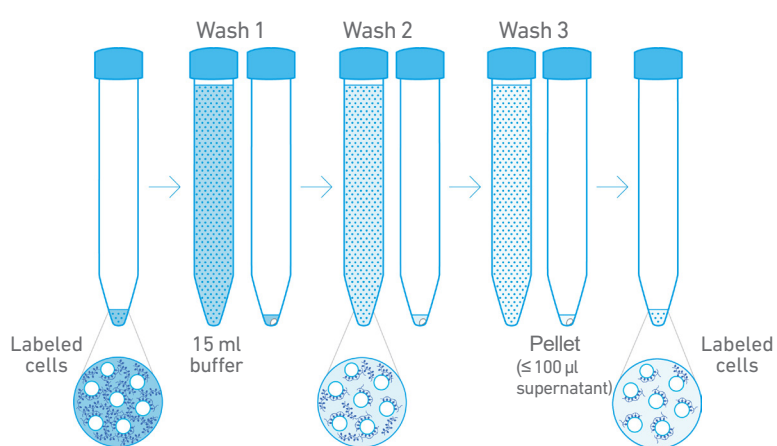
## Illustrative Overview of Wash Steps

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

### Wash Steps in 1.5-ml Tubes

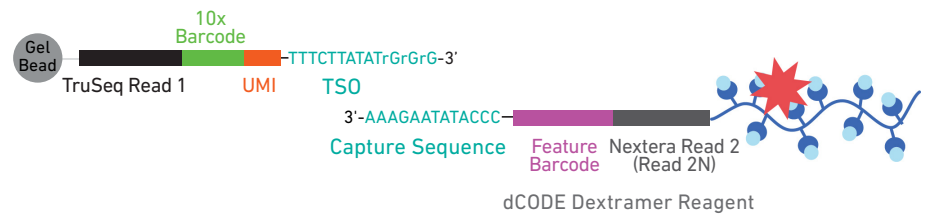


### Wash Steps in 15-ml Tubes



## Illustrative Overview of dCODE Dextramer Reagent Capture

### Single Cell V(D)J / v1.1 - Cell Surface Protein (CG000186/CG000208)



## References

- Chromium Single Cell V(D)J Reagent Kits User Guide with Feature Barcoding technology for Cell Surface Protein (Document CG000186)
- Chromium Next GEM Single Cell V(D)J Reagent Kits v1.1 User Guide with Feature Barcoding technology for Cell Surface Protein (Document CG000208)

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