DEMONSTRATED PROTOCOL

Cell Labeling with dCODE™ Dextramer® Reagents for Single Cell RNA Sequencing Protocols

with Feature Barcode technology

Overview

Multimeric MHC peptide complexes, such as dCODE™ 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 (dCODE™ Dextramer® MHC-Feature Barcode oligonucleotide conjugate) along with TotalSeq-C antibody-oligonucleotide conjugates. This document also provides guidance for enriching dCODE™ Dextramer®+ T cells by Fluorescence Activated Cell Sorting (FACS). These dCODE™ Dextramer® reagents and TotalSeq-C antibody-oligonucleotide conjugate 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 Barcode technology (CG000186, CG000208, CG000330, and CG000424).

To obtain more accurate cell calling of the analysis, cells should be labeled with both antibody-oligonucleotide conjugates and dCODE™ Dextramer® reagents. dCODE™ Dextramer® reagent only type of analyses are not supported currently.

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 Prepare fresh, maintain at 4°C	Composition
Biotin Stock Solution	100 µM D-Biotin in PBS
Resuspension Buffer	PBS + 0.04% BSA
PBS + 2% FBS (maintain at 4°C)	

Specific Reagents & Consumables

Vendor	Item	Part Number
Immudex	dCODE™ Dextramer® Reagents	-
	dCODE™ Dextramer® Reagents Controls	-
BioLegend	Human TruStain FcX (Fc Receptor Blocking Solution)	422301
	TotalSeq™-C Antibody-Oligonucleotide Conjugate (see Appendix for a list of recommended antibody-oligonucleotide conjugates)	-
	Antibodies (Fluorophore)* If enriching dCODE™ Dextramer®+ cells through FACS	-
Corning	Phosphate-Buffered Saline, 1X without Calcium and Magnesium	21-040-CV
Thermo Fisher Scientific	UltraPure Bovine Serum Albumin (BSA, 50 mg/ml)	AM2616
	Trypan Blue Stain (0.4%)	T10282
Miltenyi Biotec	MACS BSA Stock Solution (Alternative to Thermo Fisher product)	130-091-376
VWR	Fetal Bovine Serum (FBS)	97068-085
MP Biomedicals	D-Biotin (>98% purity)	194634
Equipment†		
Thermo Fisher Scientific	Countess II FL Automated Cell Counter	AMAQAF1000
	Countess II FL Automated Cell Counting Chamber Slides	C10228

†This list may not include some standard laboratory equipment.

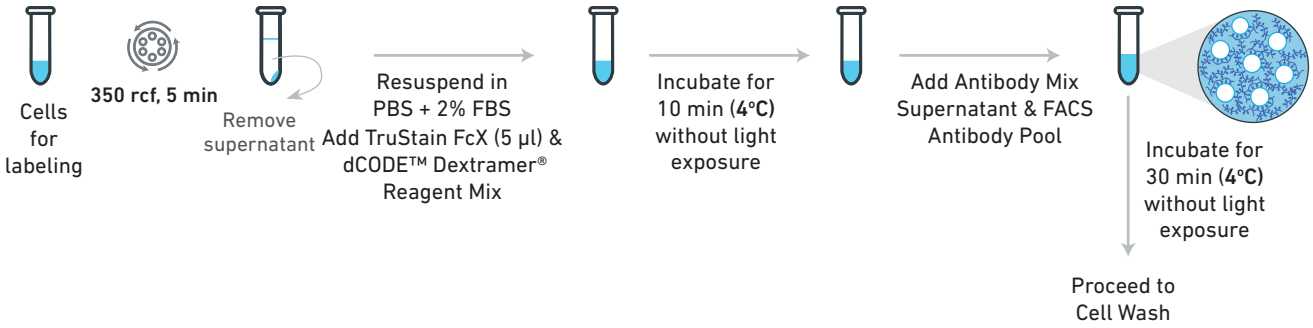
*Choose different clones than antibody-oligonucleotide conjugates.

Protocol Overview

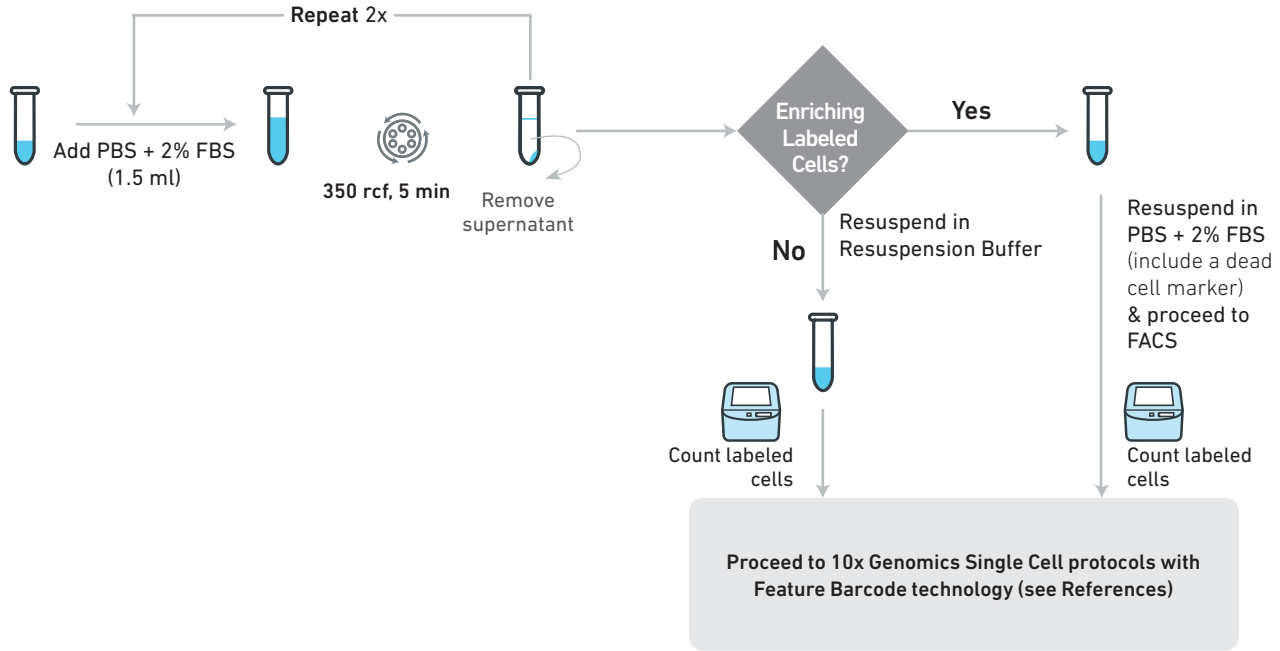
Label Cells

Prepare following as described in the Cell Labeling Protocol:

- dCODE™ Dextramer® Reagent Mix
- Antibody Mix Supernatant
- FACS Antibody Pool (if performing FACS enrichment of dCODE™ Dextramer®+ T cells)



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 can be further enriched for dCODE™ Dextramer®$^{+}$ 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

All steps can be performed in 1.5-ml microcentrifuge tubes or 15-ml centrifuge tubes.

Prepare dCODE™ Dextramer® Reagent Mix:

- Add **0.2 µl** Biotin Stock for each specific dCODE™ Dextramer® reagent to a 1.5-ml microcentrifuge tube.

  Addition of Biotin is essential to reagent performance.
- Add **2 µl** of each dCODE™ Dextramer® reagent per reaction.
- Add appropriate dCODE™ Dextramer® reagent Controls as recommended by Immudex. Gently pipette mix and maintain at **4°C**. Avoid light exposure.

Prepare Antibody Mix Supernatant:

- Add appropriately titrated amount of antibody-oligonucleotide conjugates (see Appendix) to a 1.5-ml microcentrifuge tube. Antibody titration is critical to obtain high-quality data. Depending on the total volume of cells and dCODE™ Dextramer® reagent mix, the antibody mix volume can be increased by adding PBS + 2% FBS.
- To avoid antibody aggregates, centrifuge the mix at **14,000 rcf** for **10 min** at **4°C**.
- Transfer the supernatant (containing the Antibody Mix) to a new tube and maintain at **4°C**.

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**. Avoid light exposure. See Appendix for an example of the antibodies that can be used.

This protocol was demonstrated using 3-10 x 10^6 cells. Total reaction volume for labeling is **100 µl**.

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 pellet in an appropriate volume PBS + 2% FBS. The resuspension volume depends upon the volumes of dCODE™ Dextramer® Reagent Mix, FACS Antibody Pool, and Antibody Mix.

d. Add **5 µl** (1:20 dilution of **100 µl** reaction) Human TruStain FcX. Gently pipette mix.

e. Add prepared dCODE™ Dextramer® Reagent Mix. DO NOT add FACS Antibody Pool and/or Antibody Mix at this step.

f. Incubate for **10 min** at **4°C** without light exposure.

Incubation time can be increased to 30 min when using >10 dCODE™ Dextramer® reagents.

g. Add Antibody Mix supernatant and FACS Antibody Pool at this step. If needed, add PBS + 2% FBS for a total volume of **100 µl.**

h. Incubate for **30 min** at **4°C** without light exposure.

Recommended incubation temperature for most sample types is **4°C.** However, incubation temperature is sample type dependent and should be chosen accordingly.

2. Wash Cells

Thorough washing of cells post labeling is critical to obtain high-quality data. To eliminate non-specific binding, wash steps may be performed in 1.5-ml microcentrifuge 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 1h.

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 dCODE™ 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, determine cell concentration and viability using a Countess II Automated Cell Counter or a hemocytometer and proceed **immediately** to Chromium Single Cell Immune Profiling Solutions User Guide with Feature Barcode technology (see References).

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 or a hemocytometer and proceed **immediately** to Chromium Single Cell Immune Profiling Solutions User Guide with Feature Barcode technology (see References).

Appendix

Antibody-Oligonucleotide Conjugate Panel

Use a TotalSeq-C antibody-oligonucleotide conjugate panel so that all cells can be labeled in a diverse pool of T cells. Recommended TotalSeq-C antibodies for labeling in conjunction with dCODE™ Dextramer® reagents are listed below. The list can be modified depending upon the specific experiment.

Recommended TotalSeq-C Antibody-Oligonucleotide Conjugates :

Antibody-Oligonucleotide Conjugate	Recommended Amount (µg)
CD3	0.025
CD4	0.025
CD8a	0.005
CD56	0.05
Mouse isotype control IgG1	0.05
Mouse isotype control IgG2a	0.05
Mouse isotype control IgG2b	0.05

Alternatively, the lyophilized TBNK panel from BioLegend and appropriate mouse Isotype controls may be used.

FACS Guidance

Enrich dCODE™ Dextramer®$^{+}$ 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 20% FBS to maintain cell viability. Cells should be collected either in **20 µl** volume in the collection tube/plate (96-well plate) or **200 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** at **4°C** 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 Barcode technology (see References).

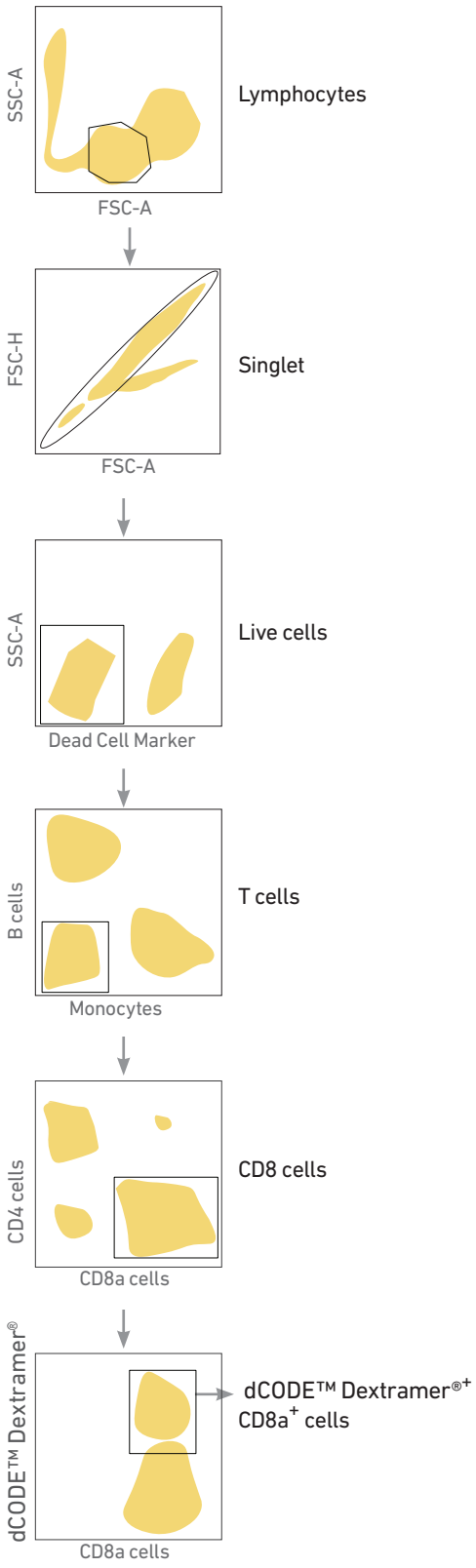
Example FACS Antibody Pool & Gating Strategy

This protocol was optimized using following fluorophore antibodies for cell sorting:

- 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

Following fluorochromes were used: BV421, PE-Cy7, BV510, FITC, and 7AAD viability dye occupying the PerCp-Cy5.5 channel

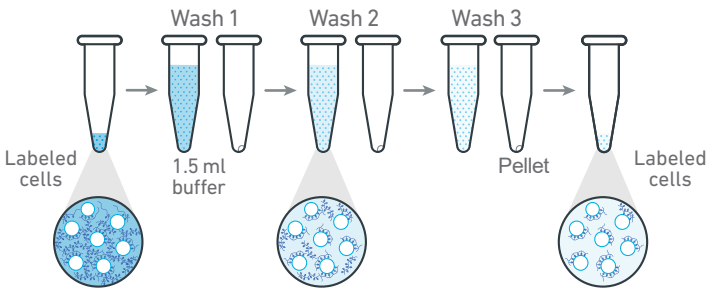
Figure 1. Example gating strategy using above mentioned antibodies is shown below:



Illustrative Overview of Wash Steps

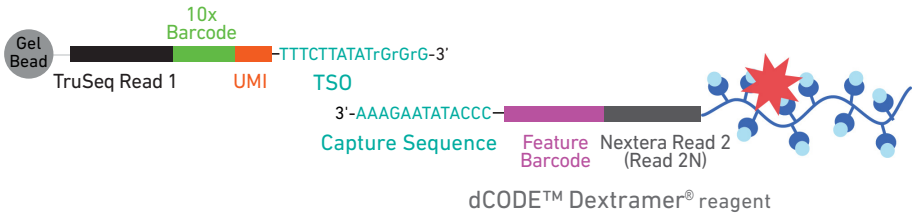
An illustrative overview of wash steps for dCODE™ Dextramer® reagent labeled cells is shown below.

Wash Steps in 1.5-ml Microcentrifuge Tubes



Illustrative Overview of dCODE™ Dextramer® Reagent Capture

Single Cell 5' v1, v1.1, and v2 - Cell Surface Protein (CG000186, CG000208, and CG000330)



References

- Chromium Next GEM Single Cell 5' HT Reagent Kits v2 (Dual Index) with Feature Barcode technology for Cell Surface Protein & Immune Receptor Mapping User Guide (Document CG000424)
- Chromium Next GEM Single Cell 5' Reagent Kits v2 (Dual Index) with Feature Barcode technology for Cell Surface Protein & Immune Receptor Mapping User Guide (Document CG000330)
- Chromium Next GEM Single Cell V(D)J Reagent Kits v1.1 with Feature Barcode technology for Cell Surface Protein User Guide (Document CG000208)
- Chromium Single Cell V(D)J Reagent Kits with Feature Barcode technology for Cell Surface Protein User Guide (Document CG000186)

Contact:
support@10xgenomics.com
10x Genomics
6230 Stoneridge Mall Road
Pleasanton, CA 94588 USA