DEMONSTRATED PROTOCOL

Cell Surface Protein Labeling for Single Cell RNA Sequencing Protocols

with Feature Barcoding technology

Overview

Cell surface proteins can be labeled using a specific protein binding molecule, such as an antibody conjugated to a Feature Barcode oligonucleotide. This protocol provides guidance for antibody-oligonucleotide conjugation and outlines cell surface protein labeling for use with:

- Chromium Single Cell 3' Reagent Kits v3 User Guide with Feature Barcoding technology for Cell Surface Protein (CG000185)
- Chromium Single Cell V(D)J Reagent Kits User Guide with Feature Barcoding technology for Cell Surface Protein (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
Labeling Buffer	PBS + 1% BSA
Resuspension Buffer	PBS + 0.04% BSA
Dextran Sulfate Solution	1% w/v (10 mg/ml) Dextran Sulfate Sodium Salt in Nuclease-free Water

Specific Reagents & Consumables

For Antibody-Oligonucleotide Conjugation		
Vendor	**Item**	**Part Number**
Expedeon	Thunder-Link PLUS Conjugation Kit	425-0300
IDT	Custom DNA Oligos (see Table 1)	-
-	100 µg Purified Azide-free Antibody (1 mg/ml)	-

For Cell Surface Protein Labeling		
Vendor	**Item**	**Part Number**
BioLegend	Human TruStain FcX (Fc Receptor Blocking Solution)	422301
	TotalSeq Antibody-Oligonucleotide Conjugate*	-
MP Biomedicals	Dextran Sulfate Sodium Salt	101516
Thermo Fisher Scientific	Dextran Sulfate Sodium Salt (alternative to MP Biomedicals product)	AC441490050
	UltraPure Bovine Serum Albumin (BSA, 50 mg/ml)	AM2616
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

*TotalSeq-B for Single Cell 3' v3 protocol with Feature Barcoding technology for Cell Surface Protein

*TotalSeq-C for Single Cell V(D)J protocol with Feature Barcoding technology for Cell Surface Protein

Protocol Overview

Option A. Custom Conjugated Antibodies

Follow manufacturer's instructions
(Thunder-Link PLUS) for conjugation and purification

Option B. Preconjugated Antibodies

BioLegend TotalSeq-B (for Single Cell 3' v3)
OR
BioLegend TotalSeq-C (for Single Cell V(D)J)

1. Label Cells

Prepare Antibody Mix
Centrifuge (**14,000 rcf, 10 min**)
Maintain Antibody Mix supernatant at 4°C

Centrifuge cells for labeling (**400 rcf, 5 min**)
Remove supernatant

↓

Resuspend pellet in Labeling Buffer (**50 µl**)

↓

Add Human TruStain FcX (**5 µl**)
Add Dextran Sulfate solution (**2 µl**)
Gently pipette mix

↓

Incubate at **4°C** (**10 min**)

↓

Add Prepared Antibody Mix supernatant

↓

Add Labeling Buffer
(for a total volume of **100 µl**)
Gently pipette mix

↓

Incubate at **4°C** (**30 min**)

2. Wash Cells

See Appendix for Labeling Buffer volumes when
working with 15-ml tubes

Wash 1: Add Labeling Buffer to cells (**1.4 ml**)

↓

Centrifuge (**400 rcf, 5 min**)
Remove supernatant

↓

Wash 2: Add Labeling Buffer to pellet (**1.5 ml**)
Gently pipette mix

↓

Centrifuge (**400 rcf, 5 min**)
Remove supernatant

Repeat 1x
(Wash 3)

↓

Add Resuspension Buffer to pellet
Gently pipette mix

↓

Determine cell concentration & viability

↓

Proceed to:

- **Chromium Single Cell 3' Reagent Kits v3 User Guide with Feature Barcoding technology for Cell Surface Protein (CG000185) OR**
- **Chromium Single Cell V(D)J Reagent Kits User Guide with Feature Barcoding technology for Cell Surface Protein (CG000186)**

Antibody-Oligonucleotide Conjugation Guidance

Choose antibody based on cell surface protein/s being labeled and a Feature Barcode oligonucleotide compatible with specific 10x Genomics protocol (Table 1) for conjugation. Alternatively, use compatible preconjugated antibodies from BioLegend or other vendors for labeling cells.

Specific Reagents for Conjugation

Vendor	Item	Part Number
Expedeon	Thunder-Link PLUS Conjugation Kit	425-0300
IDT	Custom DNA Oligos (see Table 1)	-
-	100 µg Purified Azide-free Antibody (1 mg/ml)	-
Corning	Phosphate-Buffered Saline, 1X without Calcium and Magnesium	21-040-CV

Oligonucleotide:

Use ≥10 nmole HPLC-purified and lyophilized Feature Barcode oligonucleotide for conjugation. The oligonucleotide must contain an amine group at 5'-end (5' amine modified; IDT code /5AmMC12/).

Resuspend lyophilized oligonucleotide in PBS or other compatible buffer (see Thunder-Link PLUS) at 100 µM, i.e. 10 nmole dissolved in 100 µl buffer.

DO NOT use Tris buffers for resuspension as they are not compatible with conjugation.

Conjugation:

Follow manufacturer's instructions (Thunder-Link PLUS Oligo Conjugation Kit from Expedeon) for antibody-oligonucleotide conjugation. Thunder-Link PLUS is compatible with many purified antibodies.

Antibody-Oligonucleotide Ratio:

This protocol was demonstrated using 1:3 antibody-oligonucleotide ratio for conjugation. Optimization may be needed depending on the antibodies used.

Conjugate Purification:

The antibody-oligonucleotide conjugate purification is recommended to remove any unbound oligonucleotides. Follow the Thunder-link PLUS conjugate purification protocol.

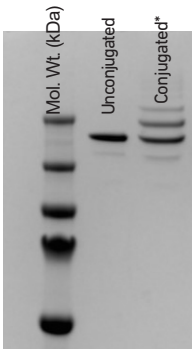
Verification of Conjugation:

Verify the conjugation by comparing the antibody-oligonucleotide conjugate with an unconjugated antibody control resolved on a non-reducing, gradient SDS-PAGE gel.

a. Run a known volume and concentration of unconjugated antibody next to a known volume of antibody-oligonucleotide conjugate on SDS-PAGE gel.

b. Estimate the conjugate concentration and calculate the degree of conjugation by comparing the respective band intensities.

Figure 1. Verification of conjugation on a 4-12% gradient SDS-PAGE gel under non-reducing conditions.



*Both unconjugated and conjugated antibodies are detected in this lane

OPTIONAL Use a BCA or Bradford Protein Assay Kit to calculate the final antibody concentration.

Table 1. Feature Barcode Oligonucleotide Sequence for Antibody Conjugation.

10x Genomics Protocol	Feature Barcode Oligonucleotide Sequence
Single Cell 3' v3 – Cell Surface Protein (CG000185)	/5AmMC12/GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNNNNNN-NNNNNNNNNNNNNNN-NNNNNNNNNGCTTTAAGGCCGGTCCTAGCAA TruSeq Read 2 — 10 nt — Feature Barcode (15 nt) — 9 nt — Capture Sequence 1
Single Cell V(D)J – Cell Surface Protein (CG000186)	/5AmMC12/CGGAGATGTGTATAAGAGACAGNNNNNNNNNN-NNNNNNNNNNNNNNN-NNNNNNNNNCCCATATAAGAAA Nextera partial Read 2 — 10 nt — Feature Barcode (15 nt) — 9 nt — Capture Sequence

See Appendix for an illustrative overview of antibody-oligonucleotide conjugate capture by 10x Gel Bead primers.

Consult Barcode Whitelist for Custom Feature Barcoding conjugates (Document CG000193), for more information.

Cell Surface Protein Labeling Protocol

Specific Reagents & Buffers

Vendor	Item	Part Number
BioLegend	Human TruStain FcX (Fc Receptor Blocking Solution)	422301
	TotalSeq Antibody-Oligonucleotide Conjugate*	-
MP Biomedicals	Dextran Sulfate Sodium Salt	101516
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*TotalSeq-B for Single Cell 3' v3 protocol with Feature Barcoding technology for Cell Surface Protein
*TotalSeq-C for Single Cell V(D)J protocol with Feature Barcoding technology for Cell Surface Protein

1. Label Cells

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

This protocol was demonstrated using 0.2-1 x 10^6 PBMCs.

a. Centrifuge cells at **400 rcf** for **5 min** at **4°C.** Use of swinging-bucket rotor is recommended for higher cell recovery.

b. Remove the supernatant without disturbing the pellet.

c. Resuspend cell pellet in **50 µl** Labeling Buffer.

d. Add **5 µl** Human TruStain FcX.

e. Add **2 µl** Dextran Sulfate Solution. Gently pipette mix.

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

g. Add the prepared Antibody Mix supernatant.

h. Add Labeling Buffer to the cell suspension to bring the total volume to **100 µl**. Gently pipette mix 10x (pipette set to 90 µl).

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

2. Wash Cells

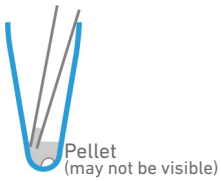
To eliminate non-specific binding with comparable efficiency, wash steps may be performed either in 1.5-ml microcentrifuge tubes or 15-ml tubes using indicated Labeling Buffer volumes. If concerned about dislodging the pellet during supernatant removal, perform wash steps in 15-ml tubes (see Appendix).

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

a. Wash by adding **1.4 ml** Labeling Buffer (for a total volume of **1.5 ml**) to the cells from step 1i.

b. Centrifuge at **400 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.

d. Using a **wide-bore** pipette tip, resuspend the cell pellet in **1.5 ml** Labeling Buffer.

e. Centrifuge at **400 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**.

h. Based on starting cell concentration and assuming ~50% cell loss, add an appropriate volume of Resuspension Buffer to obtain a cell concentration of 700-1,200 cells/µl and gently pipette mix using a **regular-bore** pipette tip until a single cell suspension is achieved.

i. Determine cell concentration and viability using a Countess II Automated Cell Counter or a hemocytometer.

j. Proceed **immediately** to:

- Chromium Single Cell 3' Reagent Kits v3 User Guide with Feature Barcoding technology for Cell Surface Protein (CG000185)

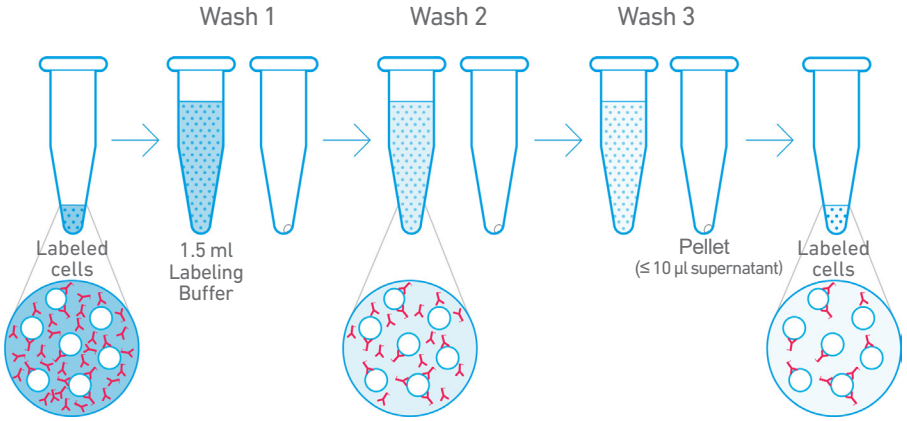
  OR

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

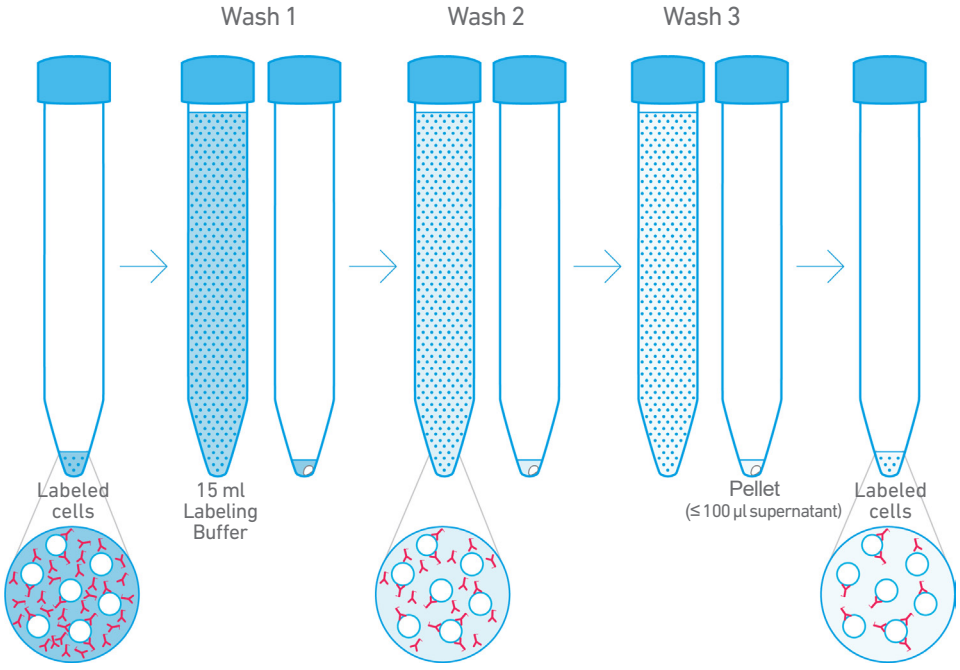
Appendix

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

Illustrative Overview of Wash Steps in 1.5-ml Tubes



Illustrative Overview of Wash Steps in 15-ml Tubes

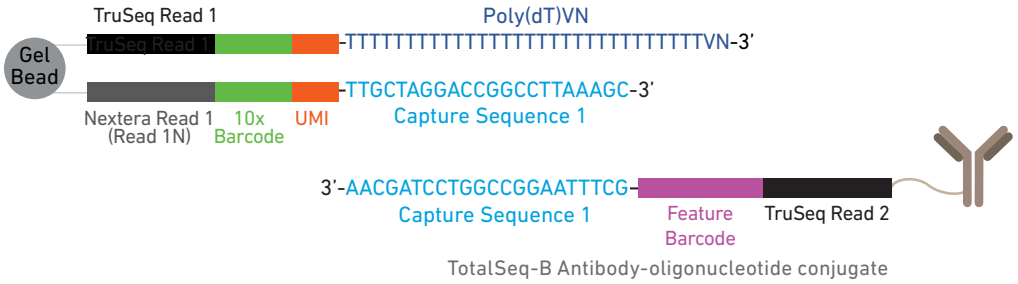


Appendix

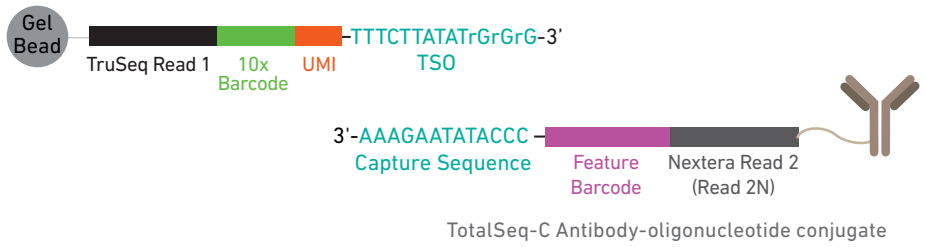
Antibody-oligonucleotide conjugate capture by protocol specific Gel Bead primers is illustrated below.

Illustrative Overview of Antibody-Oligonucleotide Conjugate Capture

Single Cell 3' v3 – Cell Surface Protein (CG000185)



Single Cell V(D)J – Cell Surface Protein (CG000186)



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