Cell Surface Protein Labeling for Single Cell RNA Sequencing Protocols *with Feature Barcode technology*

Getting Started

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 Single Cell RNA sequencing protocols with Feature Barcode technology for cell surface protein. This protocol also provides guidance for enriching labeled cells using Fluorescence Activated Cell Sorting (FACS).

Consult Demonstrated Protocol Cell Multiplexing Oligo Labeling for Single Cell RNA Sequencing Protocols with Feature Barcode technology (CG000391) for guidance on cell surface protein and Cell Multiplexing Oligo labeling.

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.

Specific Reagents & Consumables

For Antibody-Oligonucleotide Conjugation

Vendor	Item	Part Number
Abcam	Oligonucleotide Conjugation Kit	ab218260
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 Conjugates*	-
	Cell Staining Buffer	420201
	Antibodies (Fluorophore)† *If using FACS for enriching labeled cells*	-
Thermo Fisher Scientific	UltraPure Bovine Serum Albumin (BSA, 50 mg/ml)	AM2616
	Trypan Blue Stain (0.4%)	T10282
	Fetal Bovine Serum, qualified, heat inactivated	16140071
Millipore Sigma	Bovine Serum Albumin In DPBS (10%) *Alternative to Thermo Fisher product*	A1595
Miltenyi Biotec	MACS BSA Stock Solution *Alternative to Thermo Fisher product*	130-091-376
Corning	Phosphate-Buffered Saline, *1X without Calcium and Magnesium*	21-040-CV

Vendor	Item	Part Number
VWR	Fetal Bovine Serum (FBS) *Alternative to Thermo Fisher product*	97068-085
Eppendorf	DNA LoBind Tube 5.0 ml	0030108310
Equipment		
Thermo Fisher Scientific	Countess II FL Automated Cell Counter	AMQAF1000
	Countess II FL Automated Cell Counting Chamber Slides	C10228

This list may not include some standard laboratory equipment.

***TotalSeq™-B for:** Single Cell 3' v3 and v3.1 protocol with Feature Barcode technology for Cell Surface Protein

***TotalSeq™-C for:** Single Cell 5' v1, v1.1 & v2 protocol with Feature Barcode technology for Cell Surface Protein & Immune Receptor Mapping

†Choose different clones than antibody-oligonucleotide conjugates

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
Abcam	Oligonucleotide Conjugation Kit	ab218260
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 Oligonucleotide Conjugation Kit) 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 (Oligonucleotide Conjugation Kit from Abcam) for antibody-oligonucleotide conjugation. Abcam Oligonucleotide Conjugation Kit is compatible with many purified antibodies.

Table 1. Feature Barcode Oligonucleotide Sequence for Antibody Conjugation

10x Genomics Protocol	Feature Barcode Oligonucleotide Sequence
Single Cell 3' v3 & v3.1* – Cell Surface Protein *Single & dual index libraries	/5AmMC12/GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT-NNNNNNNNNN-NNNNNNNNNNNNNNN-NNNNNNNNN-GCTTTAAGGCCGGTCCTAGCAA TruSeq Read 2 — 10 nt — Feature Barcode (15 nt) — 9 nt — Capture Sequence 1
Single Cell 5' v1, v1.1 & v2 – Cell Surface Protein	/5AmMC12/CGGAGATGTGTATAAGAGACAGNNNNNNNNNN-NNNNNNNNNNNNNNN-NNNNNNNNN-CCCATATAAGAAA 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 Barcode conjugates (Document CG000193), for more information.

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

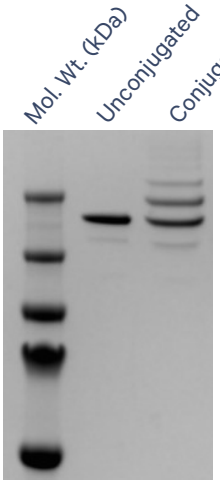
The antibody-oligonucleotide conjugate purification is recommended to remove any unbound oligonucleotides. Follow the Conjugate Purification protocol (Abcam).

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. Run a known volume and concentration of unconjugated antibody next to a known volume of antibody-oligonucleotide conjugate on SDS-PAGE gel. Estimate the conjugate concentration and calculate the degree of conjugation by comparing the respective band intensities.

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

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

Tips & Best Practices

Cell Viability

- Determine sample viability before starting the cell labeling protocol.
- Cell washing instructions are different for samples with different cell viability. See Sample Viability & Appropriate Washing Protocol for details on how to choose appropriate washing protocol.

Labeling & Wash Buffer

- Labeling or washing buffer for most sample types is PBS + 1% BSA.
- Sample types containing <70% viable cells, samples that are known to degrade quickly, or samples with very low cell input (<200,000 cells), may require a buffer containing serum.
- Keeping the cells viable is crucial for a successful experiment. FBS above 2% can lead to a slight reduction in number of genes/cell.

Centrifugation Conditions

- Centrifugation speed and time depends upon the sample type.
- Larger or fragile cell types may require slower centrifugation speeds.

Table 2. Sample Type Specific Centrifugation Conditions

Sample Type	Centrifugation Conditions
Samples containing >85% viable cells, e.g., PBMCs	300-400 rcf for 5 min
Samples containing <85% viable cells, e.g., tumor cells	150-300 rcf for 5-10 min

Optimal Antibody Concentration

- Consult Technical Note Quality Control of Cell Surface Protein Labeling using Flow Cytometry (CG000231) for guidance on how to titrate antibodies using flow cytometry for determining optimal antibody concentrations.

Sample Viability & Appropriate Washing Protocol

Before labeling cells with the antibody-oligonucleotide conjugate, determine cell viability of the sample. Cell washing instructions for the labeled cells depend upon the starting cell viability. This document provides four washing protocols for samples with different viability. Choose appropriate washing protocol. See Table 3 for a comparison of the four washing protocols.

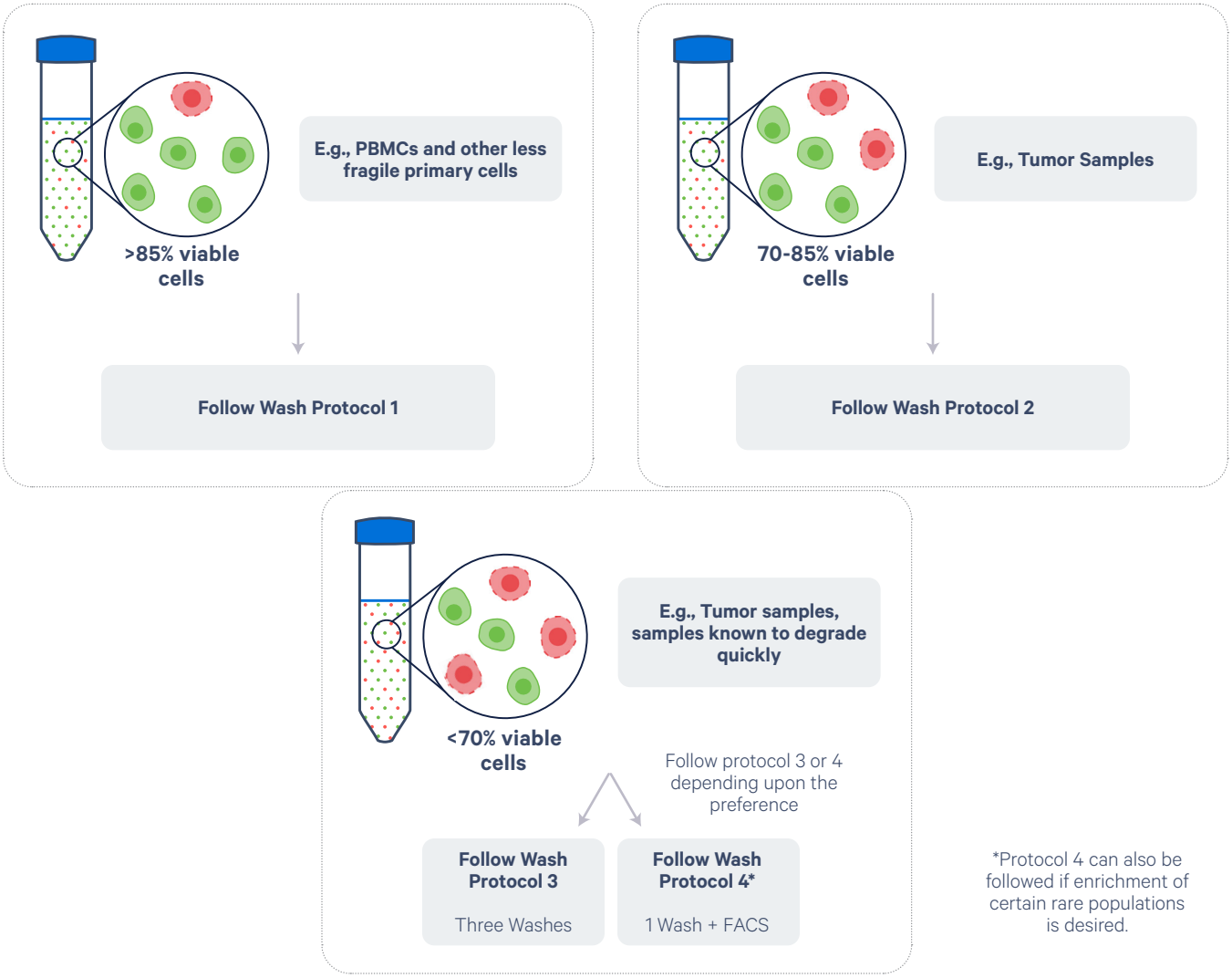


Table 3. Sample Type Specific Washing Protocols

Protocol Specifics	Wash Protocol 1 (>85% viable cells)	Wash Protocol 2 (70-85% viable cells)	Wash Protocol 3 (<70% viable cells)	Wash Protocol 4 (<70% viable cells)
Total Washes	4	3	3	1
Wash Buffer	Wash 1: PBS + 0.04% BSA Washes 2-4: PBS + 1% BSA	PBS + 1% BSA	PBS + 10% FBS	PBS + 10% FBS
Incubation in PBS	√	X	X	X
Centrifugation Conditions	300-400 rcf, 5 min, 4°C	150-300 rcf, 5-10 min, 4°C	150-300 rcf, 5-10 min, 4°C	150-300 rcf, 5-10 min, 4°C
Cleanup & Enrichment by FACS	Optional	Optional	Optional	√

Protocol Overview

Option A. Custom Conjugated Antibodies

Follow manufacturer's instructions
(Abcam Oligonucleotide Conjugation Kit)
for conjugation and purification

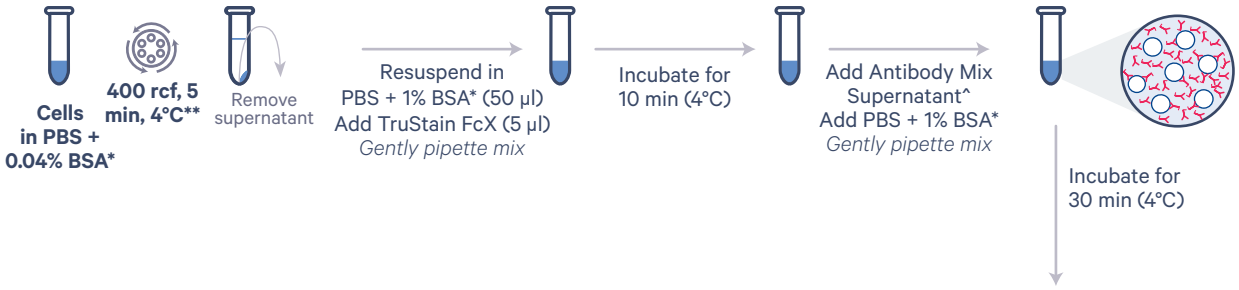
Option B. Preconjugated Antibodies

BioLegend TotalSeq™-B
OR
BioLegend TotalSeq™-C

Label Cells

Prepare following as described in the Labeling Protocol:

- Antibody Mix
- FACS Antibody Pool (if performing FACS enrichment)



Proceed to Cell Washing

Choose Wash Protocol 1, 2, 3, or 4 depending upon the initial sample viability

***For samples <70% viable cells, use PBS + 10% FBS**

**Centrifugation conditions are sample type dependent

^If performing FACS enrichment, add prepared FACS Antibody Pool

Cell Surface Protein Labeling Protocol

This protocol was optimized using TotalSeq-B/C antibody-oligonucleotide conjugates from BioLegend. The labeled cells can be enriched by FACS (see Appendix).

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

This protocol was demonstrated using 0.2-2 x 10^6 cells. Wash cells according to the appropriate 10x Genomics Demonstrated Protocol for the cell type being prepared. All steps can be performed in 5-ml centrifuge tubes, 15-ml centrifuge tubes or round-bottom FACS tubes.

Buffers – Preparation

For samples containing >70% viable cells

- Chilled (4°C): PBS + 1% BSA
- Chilled (4°C): PBS + 0.04% BSA

For samples containing <70% viable cells

- Chilled (4°C): PBS + 10% FBS

Prepare Antibody Mix Supernatant

- Add appropriate/manufacturer's recommended amount of antibody-oligonucleotide conjugates to a 1.5-ml microcentrifuge tube.
- If using a custom lyophilized antibody: Resuspend the antibody-oligonucleotide conjugates in an appropriate volume of labeling buffer.
- Centrifuge the mix at **14,000 rcf** for **10 min** at **4°C**.
- Transfer the supernatant (containing Antibody Mix) to a new tube and maintain at **4°C**.

Use TotalSeq-B for Single Cell 3' v3 and v3.1 (single & dual index) protocol and TotalSeq-C for Single Cell 5' v1, v1.1 & v2 protocol with Feature Barcode technology for Cell Surface Protein.

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.

a. Transfer cells to a new 5-ml tube and add chilled PBS + 0.04 % BSA for a total 1 ml volume. *For samples containing <70% viable cells, use chilled PBS + 10% FBS.*

b. Centrifuge cells at **4°C**. Use of swinging-bucket rotor is recommended for higher cell recovery. Centrifugation speed and time depends upon the sample type. Larger or fragile cell types may require slower centrifugation speeds. *Use Table 2 - Sample Type Specific Centrifugation Conditions for guidance.*

c. Remove the supernatant without disturbing the pellet.

d. Resuspend cell pellet in **50 µl** chilled PBS + 1% BSA. *For samples containing <70% viable cells, resuspend in chilled PBS + 10% FBS.*

e. Add **5 µl** Human TruStain FcX. Gently pipette mix.

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

g. Add the prepared Antibody Mix supernatant. If also performing FACS enrichment, add FACS antibody pool.

h. Add chilled PBS + 1% BSA to the cells to bring the total volume to **100 µl**. Gently pipette mix 10x (pipette set to 90 µl). *For samples containing <70% viable cells, add chilled PBS + 10% FBS.*

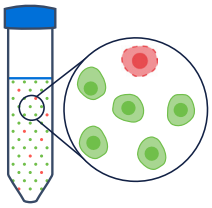
i. Incubate for **30 min** at **4°C**. If using FACS antibodies, incubate without light exposure.

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

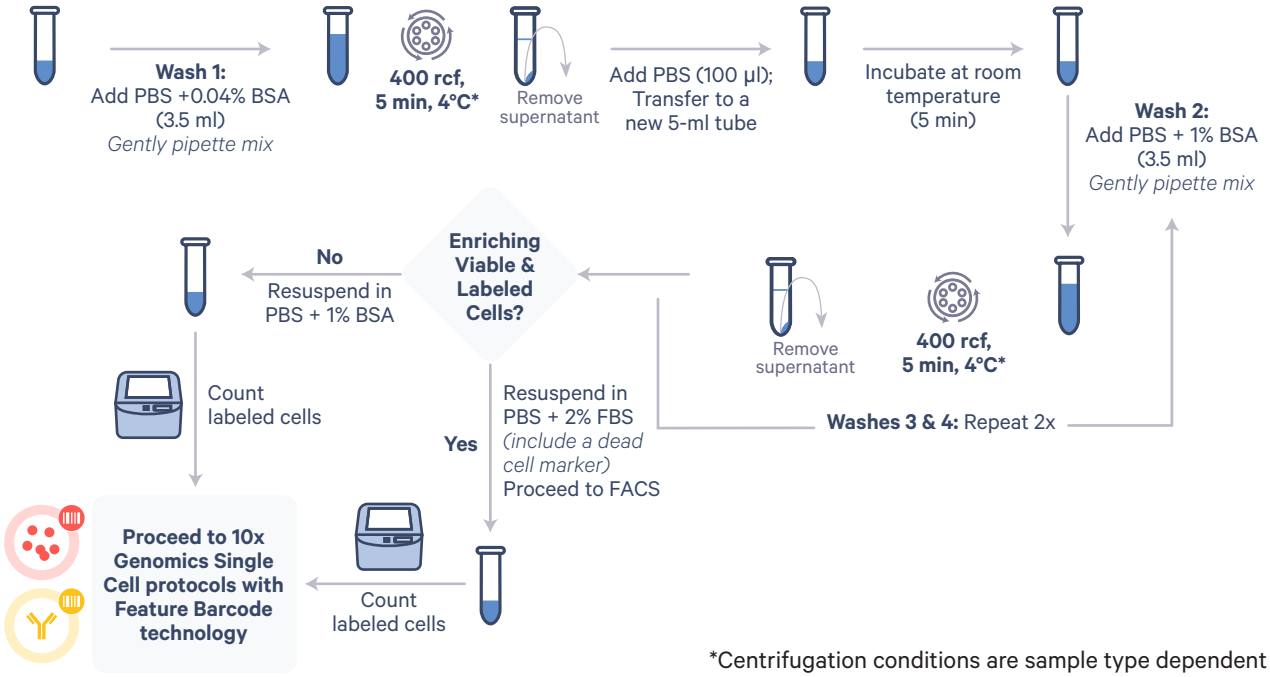
j. Proceed to cell washing.
Cell washing protocol and wash & resuspension buffers depends upon the sample. Choose appropriate protocol based on starting sample viability.

Protocol Overview: Wash Protocol 1

for Samples with >85% Viable Cells

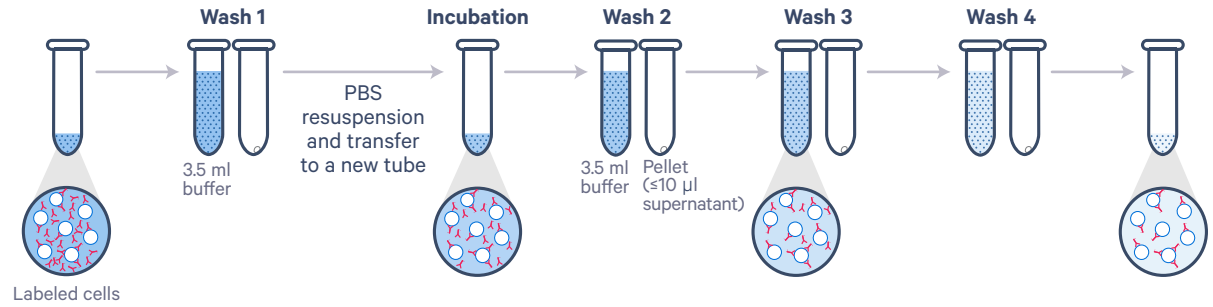


Wash Protocol 1 (>85% viable cells)



*Centrifugation conditions are sample type dependent

Illustrative Overview of the Washes for Wash Protocol 1



Cell Washing Protocol 1

for Samples with >85% Viable Cells

Thorough washing of cells post labeling is critical to obtain high-quality data. Optimization of centrifugation speed/time may be needed based on cell type.

Buffers – Preparation

- Chilled (4°C): PBS + 0.04% BSA
- Chilled (4°C): PBS + 1% BSA

a. Wash 1:
Wash by adding **3.5 ml** chilled PBS + 0.04% BSA to the labeled cells. Gently pipette mix.

b. Centrifuge at **4°C**. Centrifugation speed and time depends upon the sample type. Use Table 2 for guidance.

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. Resuspend the pellet in **100 µl** room temperature PBS and transfer to a new 5-ml tube. Incubate for **5 min** at **room temperature**.

e. Wash 2:
Using a pipette tip, resuspend the pellet or cells in **3.5 ml** chilled PBS + 1% BSA.

f. Centrifuge at **4°C**. Centrifugation speed and time depends upon the sample type. Use Table 2 for guidance.

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

h. Washes 3 & 4:
Repeat e - g 2x for a total of four washes.

i. OPTIONAL
For enrichment of labeled and viable cells by FACS:

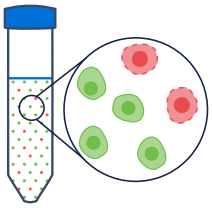
- Based on starting concentration and assuming ~50% cell loss, add an appropriate volume chilled PBS + 2% FBS (including a dead cell marker) to obtain a final cell concentration of 5-10 x 10^6 cells/ml.
- Proceed to FACS (see FACS Guidance).
- After FACS, determine cell concentration and viability using a Countess II Automated Cell Counter or a hemocytometer.
- Proceed **immediately** to relevant Chromium Single Cell RNA Sequencing protocols with Feature Barcode technology (see References).

j. If not performing FACS:

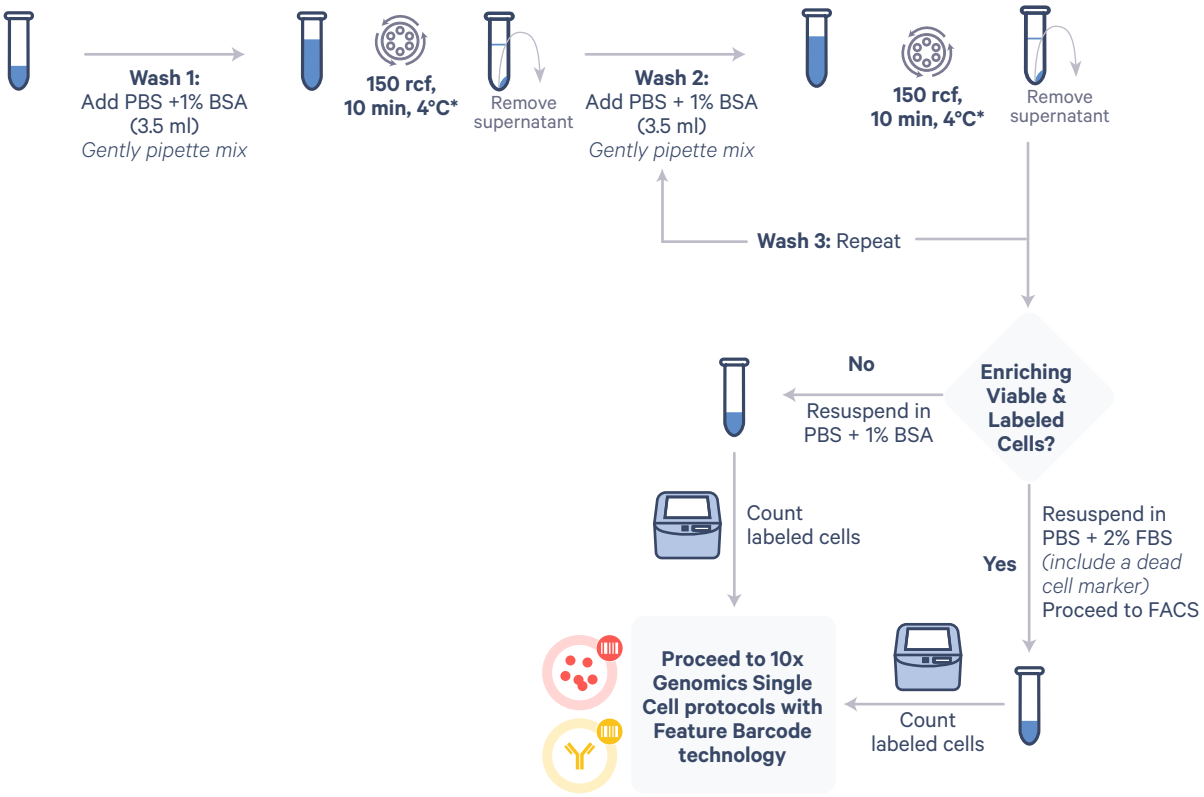
- Based on starting concentration and assuming ~50% cell loss, add an appropriate volume chilled PBS + 1% BSA to obtain a concentration of 700-1,200 cells/µl.
- Determine cell concentration and viability using a Countess II Automated Cell Counter or a hemocytometer.
- Proceed **immediately** to relevant Chromium Single Cell RNA Sequencing protocols with Feature Barcode technology (see References).

Protocol Overview: Wash Protocol 2

for Samples with 70-85% Viable Cells

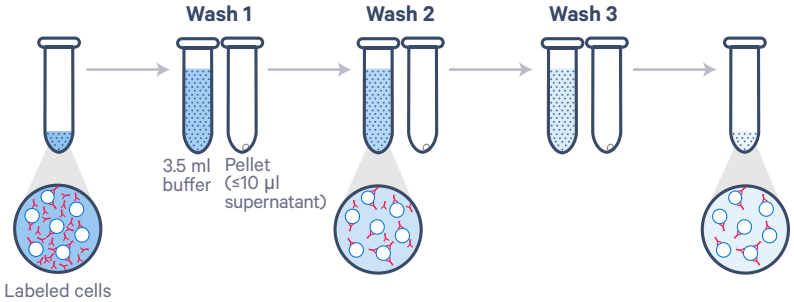


Wash Protocol 2 (70-85% viable cells)



*Centrifugation conditions are sample type dependent

Illustrative Overview of the Washes for Wash Protocol 2



Cell Washing Protocol 2

for Samples with 70-85% Viable Cells

Thorough washing of cells post labeling is critical to obtain high-quality data. Optimization of centrifugation speed/time may be needed based on cell type.

Buffers – Preparation

- Chilled (4°C): PBS + 1% BSA

a. Wash 1:
Wash by adding **3.5 ml** chilled PBS + 1% BSA to the labeled cells. Gently pipette mix.

b. Centrifuge at **4°C**. Centrifugation speed and time depends upon the sample type. Use Table 2 for guidance.

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. Wash 2:
Using a pipette tip, resuspend the pellet or cells in **3.5 ml** chilled PBS + 1% BSA and transfer to a new 5-ml tube.

e. Centrifuge at **4°C**. Centrifugation speed and time depends upon the sample type. Use Table 2 for guidance.

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

g. Wash 3 :
Using a pipette tip, resuspend the pellet or cells in **3.5 ml** chilled PBS + 1% BSA.

h. Centrifuge at **4°C**. Centrifugation speed and time depends upon the sample type. Use Table 2 for guidance.

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

j. OPTIONAL
For enrichment of labeled and viable cells by FACS:

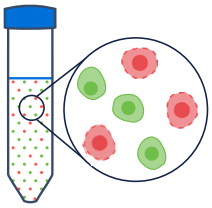
- Based on starting concentration and assuming ~50% cell loss, add an appropriate volume chilled PBS + 2% FBS (including a dead cell marker) to obtain a final cell concentration of 5-10 x 10^6 cells/ml and proceed to FACS (see FACS Guidance).
- After FACS, determine cell concentration and viability using a Countess II Automated Cell Counter or a hemocytometer.
- Proceed **immediately** to relevant Chromium Single Cell RNA Sequencing protocols with Feature Barcode technology (see References).

k. If not performing FACS:

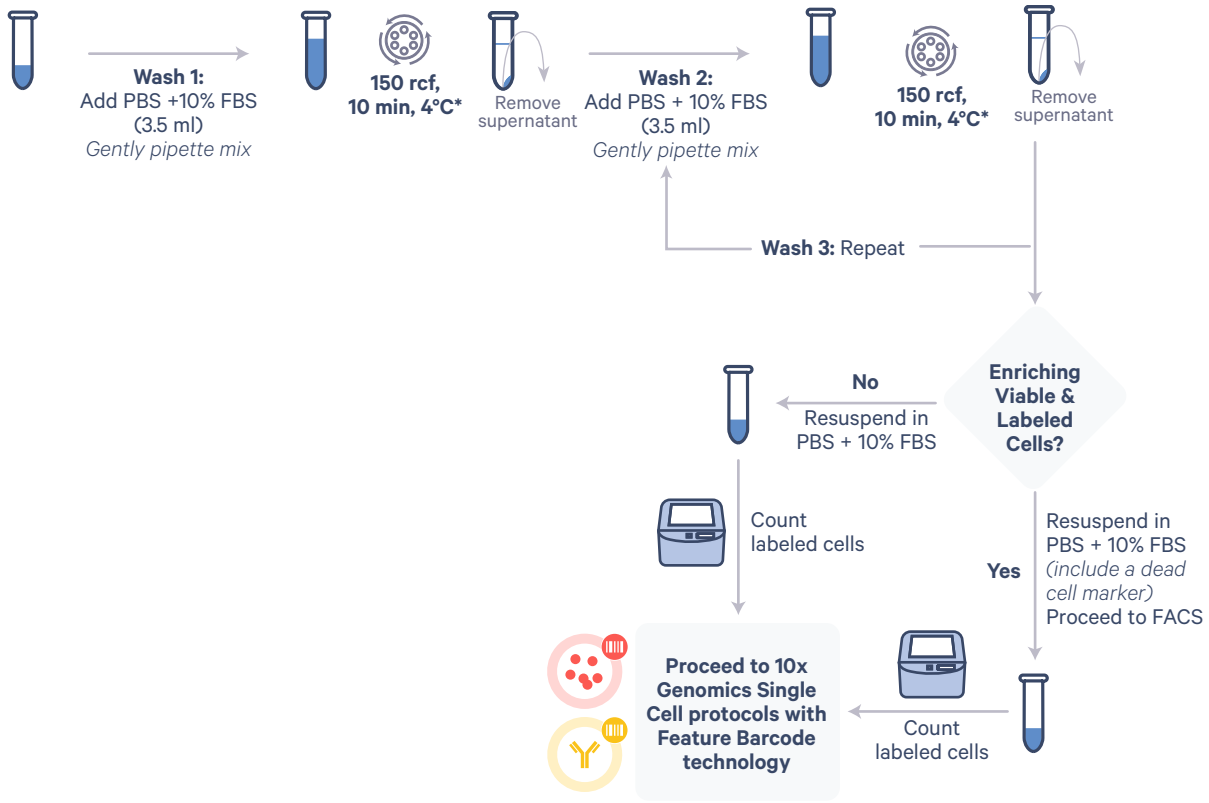
- Based on starting concentration and assuming ~50% cell loss, add an appropriate volume chilled PBS + 1% BSA to obtain a concentration of 700-1,200 cells/µl.
- Determine cell concentration and viability using a Countess II Automated Cell Counter or a hemocytometer .
- Proceed **immediately** to relevant Chromium Single Cell RNA Sequencing protocols with Feature Barcode technology (see References).

Protocol Overview: Wash Protocol 3

for Samples with <70% Viable Cells

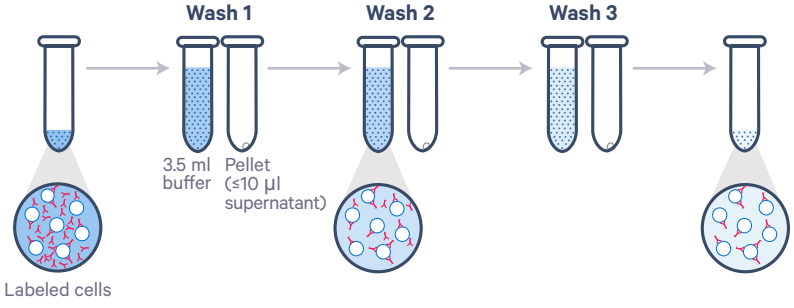


Wash Protocol 3 (<70% viable cells)



*Centrifugation conditions are sample type dependent

Illustrative Overview of the Washes for Wash Protocol 3



Cell Washing Protocol 3

for Samples with <70% Viable Cells

Thorough washing of cells post labeling is critical to obtain high-quality data. Optimization of centrifugation speed/time may be needed based on cell type.

Buffers – Preparation

- Chilled (4°C): PBS + 10% FBS

a. Wash 1:
Wash by adding **3.5 ml** chilled PBS + 10% FBS to the labeled cells. Gently pipette mix.

b. Centrifuge at **4°C**. Centrifugation speed and time depends upon the sample type. Use Table 2 for guidance.

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. Wash 2:
Using a pipette tip, resuspend the pellet or cells in **3.5 ml** chilled PBS + 10% FBS and transfer to a new 5-ml tube.

e. Centrifuge at **150-300 rcf** for **5-10 min** at **4°C**. Centrifugation speed and time depends upon the sample type. Larger or fragile cell types may require slower centrifugation speeds.

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

g. Wash 3 :
Using a pipette tip, resuspend the pellet or cells in **3.5 ml** chilled PBS + 10% FBS.

h. Centrifuge at **150-300 rcf** for **5-10 min** at **4°C**. Centrifugation speed and time depends upon the sample type. Use Table 2 for guidance.

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

j. OPTIONAL
For enrichment of labeled and viable cells by FACS:

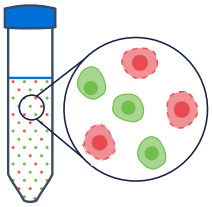
- Based on starting concentration and assuming ~50% cell loss, add an appropriate volume chilled PBS + 10% FBS (including a dead cell marker) to obtain a final cell concentration of 5-10 x 10^6 cells/ml.
- Proceed to FACS (see FACS Guidance).
- After FACS, determine cell concentration and viability using a Countess II Automated Cell Counter or a hemocytometer.
- Proceed **immediately** to relevant Chromium Single Cell RNA Sequencing protocols with Feature Barcode technology (see References).

k. If not performing FACS:

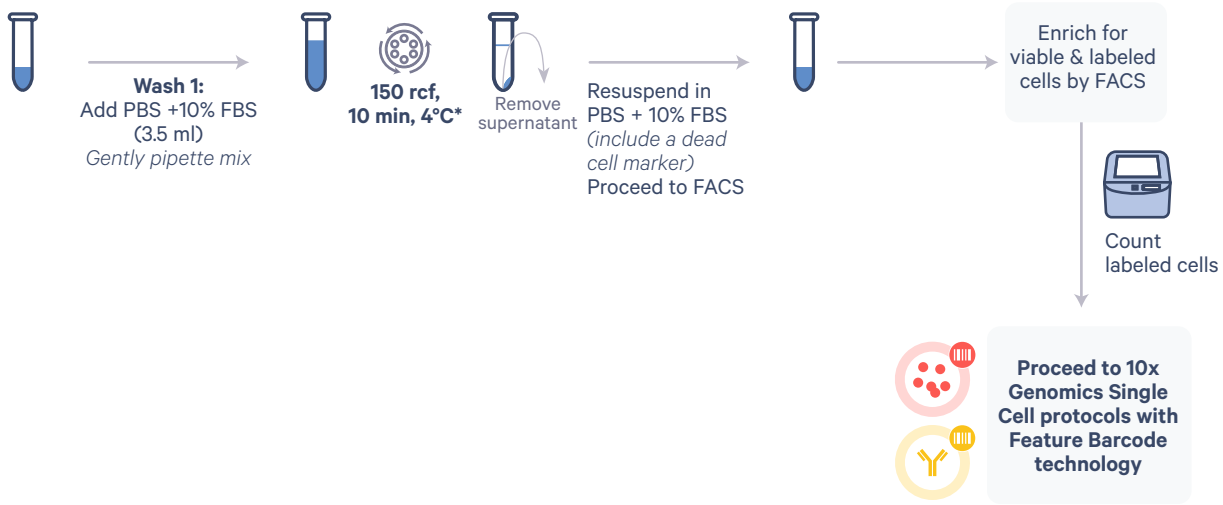
- Based on starting concentration and assuming ~50% cell loss, add an appropriate volume chilled PBS + 10% FBS to obtain a concentration of 700-1,200 cells/µl.
- Determine cell concentration and viability using a Countess II Automated Cell Counter or a hemocytometer.
- Proceed **immediately** to relevant Chromium Single Cell RNA Sequencing protocols with Feature Barcode technology (see References).

Illustrative Overview: Wash Protocol 4

for Samples with <70% Viable Cells

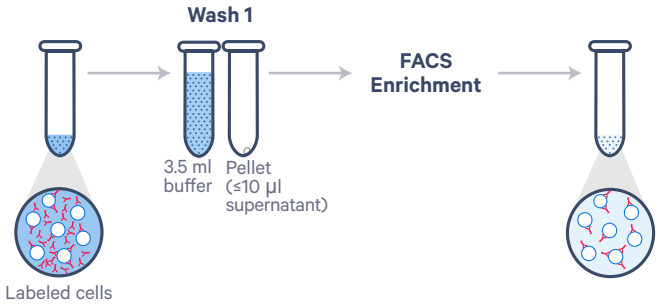


Wash Protocol 4 (<70% viable cells)



*Centrifugation conditions are sample type dependent

Illustrative Overview of the Washes for Wash Protocol 4



Cell Washing Protocol 4

for Samples with <70% Viable Cells

Thorough washing of cells post labeling is critical to obtain high-quality data. Optimization of centrifugation speed/time may be needed based on cell type.

Buffers – Preparation

- Chilled (4°C): PBS + 10% FBS

a. Wash by adding **3.5 ml** chilled PBS + 10% FBS to the labeled cells. Gently pipette mix.

b. Centrifuge at **150-300 rcf** for **5-10 min** at **4°C**. Centrifugation speed and time depends upon the sample type. 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. Enrichment of labeled and viable cells by FACS:

- Based on starting concentration and assuming ~20-30% cell loss, add an appropriate volume chilled PBS + 10% FBS (including a dead cell marker) to obtain a final cell concentration of 5-10 x 10^6 cells/ml.
- Proceed to FACS (see FACS Guidance).
- After FACS, determine cell concentration and viability using a Countess II Automated Cell Counter or a hemocytometer.
- Proceed **immediately** to relevant Chromium Single Cell RNA Sequencing protocols with Feature Barcode technology (see References).

Appendix

FACS Guidance

Enrich labeled cells using FACS prior to library generation to enable identification of rare subpopulations.

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 in 300 µl volume in a 5-ml tube. Use an optimal buffer for fragile cells to maintain a high cell viability.

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 150-300 rcf for 10 min at 4°C and by removing the supernatant. Use of a longer centrifugation time is recommended post FACS.

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

Once sorting is complete, proceed immediately to relevant Chromium Single Cell RNA Sequencing protocols with Feature Barcode technology (see References).

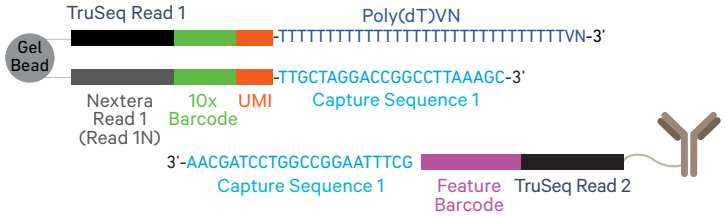
Illustrative Overview of Antibody-Oligonucleotide Conjugate Capture

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

Illustrative Overview of Antibody-Oligonucleotide Conjugate Capture

Single Cell 3' v3 & v3.1* – Cell Surface Protein

*single & dual index libraries



TotalSeq-B Antibody-oligonucleotide conjugate

Single Cell 5' v1, v1.1 & v2 – Cell Surface Protein

TotalSeq-C Antibody-oligonucleotide conjugate

Compatible Primers

To generate Cell Surface Protein libraries with TotalSeq-B/C, use the primers indicated below.

Antibody-Oligonucleotide Conjugate	Compatible Primers	10x PN
TotalSeq-B	Feature cDNA Primers 2	2000097
TotalSeq-C	SC5' Feature cDNA Primer/Feature cDNA Primer 4	2000119/ 2000277

If generating libraries with TotalSeq™-A, an additive primer is required for successful amplification (not provided by 10x Genomics). See Cite Seq Protocols and BioLegend for details.

References

This protocol provides guidance for antibody-oligonucleotide conjugation and outlines cell surface protein labeling for use with:

Single Cell 3' v3 & v3.1

- Chromium Single Cell 3' Reagent Kits v3 with Feature Barcode technology for Cell Surface Protein User Guide (CG000185)
- Chromium Next GEM Single Cell 3' Reagent Kits v3.1 with Feature Barcode technology for Cell Surface Protein User Guide (CG000206)
- Chromium Next GEM Single Cell 3' v3.1 (Dual Index) with Feature Barcode technology for Cell Surface Protein User Guide (CG000317)
- Chromium Next GEM Single Cell 3' LT Reagent Kits v3.1 (Dual Index) with Feature Barcode technology for Cell Surface Protein (CG000400)
- Chromium Next GEM Single Cell 3' HT Reagent Kits v3.1 (Dual Index) with Feature Barcode technology for Cell Surface Protein (CG000417)

Single Cell 5' v1, v1.1 & v2

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

Document Revision Summary

Document Number	CG000149
Title	Cell Surface Protein Labeling for Single Cell RNA Sequencing Protocols with Feature Barcode technology
Revision	Rev C to Rev D
Revision Date	November 2021
General Changes	Updated for general minor consistency of language and terms throughout
Specific Changes	Added Tips & Best Practices Section Added three additional cell washing protocols for samples with different viability

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