Plate-based Sample Preparation for Single Cell RNA Sequencing

Introduction

Chromium Single Cell Gene Expression solutions enable the generation of dual index libraries to study gene expression profiles, cell surface protein expression, and/or CRISPR screening in million-cell experiments. Single cell samples may be multiplexed with the 10x Genomics 3' CellPlex Kit, which provides a species-agnostic sample multiplexing solution through the use of a set of 12 Feature Barcode oligonucleotides, each conjugated to a lipid.

This protocol provides guidance for labeling cells with antibody-oligonucleotide conjugates and 3' CellPlex Cell Multiplexing Oligos (CMOs) in a high throughput and/or low sample input (200,000 cells for suspension cells, 100,000 cells for adherent cells), plate-based format.

Prior experience working with 96-well plate formats is highly encouraged. Pilot experiments are advised to minimize cell loss at each wash step and to gain familiarity with multichannel pipettes. Ensure that 300 µl volumes fit in the 96-well plate used for the experiment. Recommended plates are listed in the Specific Reagents and Consumables table.

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Additional Guidance

Consult Demonstrated Protocol Cell Preparation Guide (Document CG000053) for Tips & Best Practices on handling cells, Technical Note Guidelines on Accurate Target Cell Counts (Document CG000091) for determining accurate cell counts, Technical Note Best Practices to Minimize Chromium Next GEM Chip Clogs and Wetting Failures (Document CG000479) for information on preventing chip related failures, and Demonstrated Protocol Cell Multiplexing Oligo Labeling for Single Cell RNA Sequencing Protocols with Feature Barcode Technology (Document CG000391) for additional Tips & Best Practices.

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.

Cell Sourcing

- All cells were acquired from AllCells, ATCC, DLS, C&M Lab Pro, and iQ Biosciences.
- 3' CellPlex Kit Set A (PN-1000261) was used for cell multiplexing. Cell Multiplexing Oligos are supplied at **-20°C**.

Preparation-Buffers

Media/Buffers	Temperature
PBS + 1% BSA	Chilled (4°C)
PBS + 10% FBS	Chilled (4°C)

Specific Reagents and Consumables

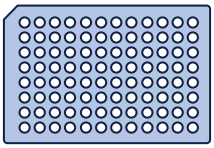
Vendor	Item	Part Number
10x Genomics	3' CellPlex Kit Set A	1000261
Rainin	Pipet-Lite Pipette Multi L12-1200XLS+	17014497
Greiner Bio-One	Microplate, 96 well, pp, v-bottom, (chimney well), natural	651201
Thermo Fisher Scientific	UltraPure Bovine Serum Albumin (BSA, 50 mg/ml)	AM2616
	Trypan Blue Stain (0.4%)	T10282
	Fetal Bovine Serum, qualified, heat inactivated	16140071
	Trypsin-EDTA	25200056
Fisher Scientific	Gast Oil-Less Diaphragm-Type Pressure/Vacuum Pump	01-092-29
	Round-Bottom Polystyrene Test Tubes	14-959-5
Corning	Phosphate-Buffered Saline, 1X without Calcium and Magensium	21-040-CV
	Costar Assay Plate, 96 Well, Clear Round Bottom, With Lid, Non-treated Polystyrene	3788
Millipore Sigma	Chemical Duty Pump, 115 V/60 Hz	WP6111560
	Bovine Serum Albumin	A1595
SP Bel-Art	Flowmi 40 micron cell strainers for 1000 microliter pipette tips	13680-0040
Miltenyi Biotec	MACS BSA Stock Solution	130-091-376
Eppendorf	DNA LoBind Tube, 5.0 mL	0030108310

General Protocol Overview

General Protocol Steps

Step 1: Preparing

Prepare Cells



and/or

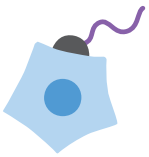
Prepare CMOs

Step 2: Labeling

Antibody Labeling

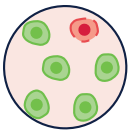
and/or

CMO Labeling



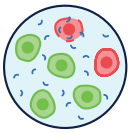
Step 3: Washing

Wash Protocol A



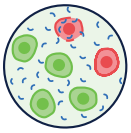
and/or

Wash Protocol B



and/or

Wash Protocol C



Tips & Best Practices

Tips & Best Practices section includes additional guidance

Signifies critical step requiring accurate execution

Troubleshooting section includes additional guidance

Sample Preparation Guidelines

This protocol was demonstrated using several primary cell types including Peripheral Blood Mononuclear Cells (PBMCs), Dissociated Tumor Cells (DTCs), freshly cultured adherent/suspension cell lines using the following protocols:

- Fresh Frozen Human Peripheral Blood Mononuclear Cells for Single Cell RNA Sequencing (Document CG00039)
- Thawing Dissociated Tumor Cells for Single Cell RNA Sequencing (Document CG000233)

Sample Quality

- Use high-quality single cell suspensions that can withstand the CMO labeling and washing steps.
- Use single cell suspensions with >80% (ideally >90%) viability. If one or more samples in the pool has lower viability, follow wash protocol B (tube-based wash), or wash protocol C (tube-based wash with fluorescence activated cell sorting (FACS)) after CMO labeling to increase viability.
- Cell Multiplexing data can be severely impacted by low cell viability, even if the single-cell behavior of the gene expression data is only mildly impacted.

CMO Tags

- Up to 12 CMO labeled samples can be pooled per well on a 10x Genomics chip. Ensure that a different CMO tag is used for each sample in the pool.
- For optimal multiplet detection and optimal signal-to-noise ratios, pool samples at 1:1 ratios after CMO labeling. The 3' CellPlex assay supports sample mixing from 50:50 to 5:95 ratios, although optimal multiplet detection occurs when samples are mixed in equal ratios.
- DO NOT pool CMO-labeled samples with unlabeled samples.

Centrifugation & Pellet Resuspension

- Use of a swinging-bucket rotor is recommended for higher cell recovery. Refer to Table 1 below for example conditions.
- For additional information, refer to Technical Note Best Practices to Minimize Chromium Next GEM Chip Clogs and Wetting Failures (Document CG000479)

Table 1: Example Sample Type Specific Centrifugation Conditions

Sample Type	Centrifugation Conditions
PBMCs	300-400 rcf for 5 min
DTCs (Post debris removal)	300 rcf for 5-10 min

Number of Input Cells

- This protocol is only supported for cell inputs of at least 10,000 cells per sample and up to 100,000 adherent cells or 200,000 cells in suspension per sample.

Wash & Resuspension Buffer

- When working with a cell type that does not have optimal viability in PBS + 1% BSA, replace PBS with most types of cell culture media and/or replace 1% BSA with 10% serum to maximize viability.
- If BSA is used, do not use less than 1% BSA. This may lead to increased background in Cell Multiplexing data. Refer to Table 2 for example wash buffers.

Table 2: Example Wash Buffers

	Culture Media + 1% BSA	PBS + 1% BSA	PBS + 10% FBS
PBMCs	⊘	✓	✓
B/T Cells and Splenocytes *Mammalian*	⊘	✓	✓
Human DTCs	⊘	⊘	✓
E18 Neuronal Cells	✓	⊘	⊘
Human and Mouse Cell Lines *Cryopreserved or Freshly Cultured*	✓	✓	✓

Debris

- Samples should be free from debris. Samples with excessive debris are not recommended as debris can contribute to high background in cell multiplexing data. If samples have high levels of debris, FACS must be performed.

Wash Steps & Supernatant Removal

- Following the appropriate wash protocol for cells post-labeling is critical to obtain high quality data and to remove background from unbound CMOs. Choose the appropriate wash protocol based on starting viability and desired level of debris clean up. Consult Table 3 for more information on wash protocol differences.
- Wash Protocol A is plate-based, while Wash Protocols B and C wash cells in 5-ml tubes. These large volume washes may be preferred if high amounts of debris are anticipated or if samples are very fragile. Wash Protocol B is a large volume wash without FACS. Wash Protocol C includes only a quench wash after CMO labeling, followed by FACS.
- After each wash step in Wash Protocol A, remove all supernatant using a vacuum pump without touching the bottom of the tube to avoid dislodging the cell pellet.
- Leaving behind excess supernatant may decrease the separation between signal and noise and lead to a large decrease in the fraction of CMO reads usable.
- In samples with lower viability (<80%) or excess debris, insufficient washing may result in a complete lack of separation between signal and noise. Wash Protocol C is recommended for these sample types, as it removes excess debris via FACS.

Time After Labeling

- Work efficiently and avoid letting cells sit for extended periods (either post-labeling or post-pooling), as allowing cells to sit may decrease sample quality and severely impact CellPlex data even if the single-cell behavior of the Gene Expression data is only mildly impacted.
- If a sample pool sits on ice for an extended period of time, perform FACS to decrease background noise in CellPlex data.
- Pool cells within 30 min of CMO labeling and washing and load cells within 30 min onto the 10x Genomics chip or proceed to FACS to enrich for viable cells.
- Keep cells on ice or 4°C at all times after CMO labeling and during/after FACS, as leaving cells at room temperature may increase background noise in CellPlex data.
- Use chilled Wash & Resuspension Buffers and perform centrifugation steps at 4°C

Choosing a Wash Protocol

Overview

Using the appropriate wash buffer for cells post-labeling is critical to obtaining high quality data and minimize background noise from unbound CMOs. Choosing a wash buffer depends on a number of sample characteristics:

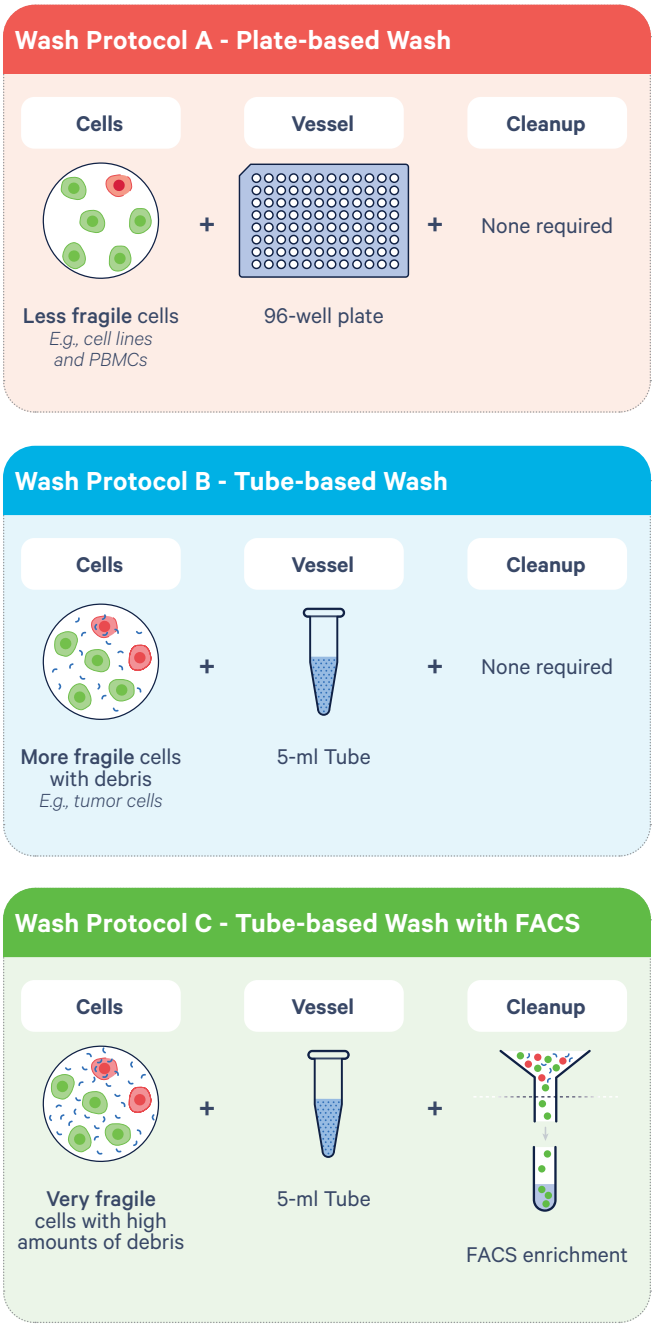
- Cell fragility
- Desired format (plate vs. tube)
- Amount of cleanup required

More information can be found in Chapter 3: Washing Cells

1.1 Preparation - Buffers

Buffer should be chosen based on what is optimal for the sample.

Media/Buffers	Composition
Complete Growth Medium *maintain at 37°C*	10% FBS in cell culture media (e.g., RPMI/DMEM)
PBS + 0.04% BSA *maintain at 4°C*	
PBS + 2% FBS *maintain at 4°C*	



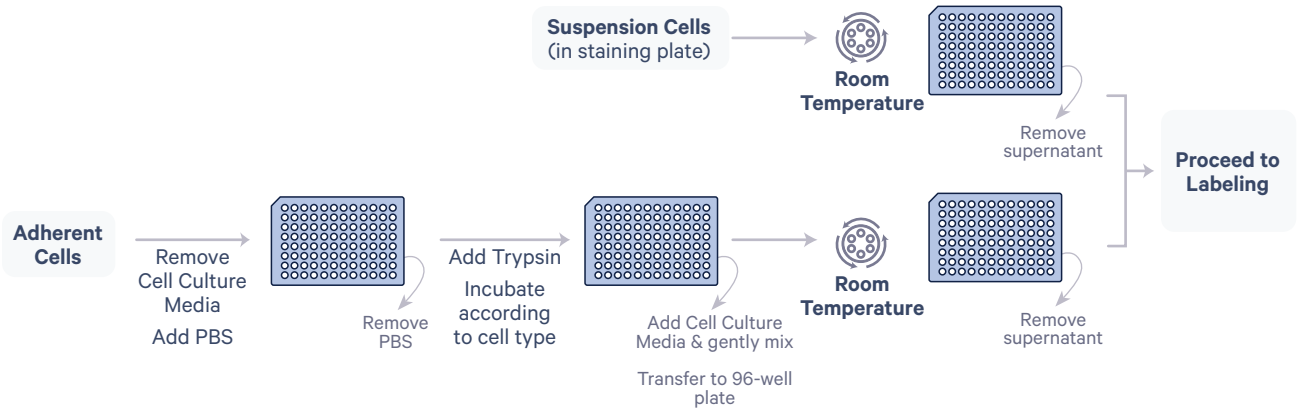
1. Cells and Cell Multiplexing Oligo Preparation

Overview

This chapter provides guidance on transfering cells to a 96-well plate and preparing CMOs. If CMO labeling is not desired, proceed directly to Chapter 2: Labeling after preparing cells.

1.1 Cell Preparation Protocol

Cell Preparation



*Centrifugation speed and time is sample dependent

Protocol Steps

This protocol was demonstrated using 10,000 – 200,000 cells per well. Use 10,000 – 200,000 cells per well for cells in suspension, or 10,000 – 100,000 cells per well for adherent cells.

a. Transfer cells to a 96-well plate, with the same number of cells in each well. If transferring adherent cells, wash cells 1x with PBS, trypsinize, add cell culture media, and pipette mix 7-10x. Transfer cell suspension into a v or round-bottom staining plate.

b. Centrifuge cells at **room temperature.** Use of a swinging-bucket rotor is recommended for higher cell recovery. Centrifugation speed and time depends upon the sample type. Refer to Table 1 on page 4.

1.2 Cell Multiplexing Oligos Preparation Protocol

If Cell Multiplexing Oligo labeling is not desired, proceed directly to step 2.1.

- Cell Multiplexing Oligos are supplied at -20°C. Cell Multiplexing Oligos may be prepared in any of the following ways.

Full Cell Multiplexing Oligo Plate Preparation

a. During cell preparation, thaw Cell Multiplexing Oligos at **room temperature**. Vortex **5 sec** at maximum speed and centrifuge briefly for **5 sec**. The 3' CellPlex Kit contains 12 Cell Multiplexing Oligos, one for each column of a 96-well plate. Two 3' CellPlex Kits are required for an entire 96-well plate.

b. Dispense **105 µl of** Cell Multiplexing Oligo into its appropriate column according to the Figure 1 below. Cell Multiplexing Oligo tubes will contain a small excess of reagent after dispensing **105 µl** into into four wells.

c. Seal plate with a plate sealer and leave at **room temperature** until use. Plate should be prepared shortly before use.

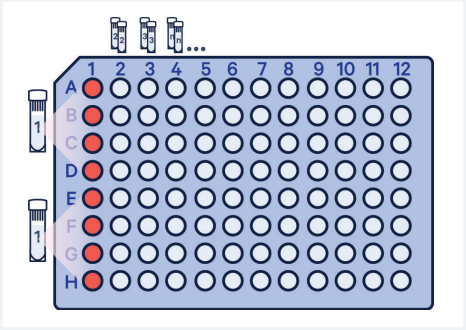


Figure 1. Cell Multiplexing Oligos are distributed in columns (two tubes per column) in the Cell Multiplexing Oligo plate.

Partial Cell Multiplexing Oligo Plate Preparation

a. During cell preparation, thaw Cell Multiplexing Oligo at **room temperature**. Vortex **5 sec** at maximum speed and centrifuge briefly for **5 sec**. The 3' CellPlex Kit contains 12 Cell Multiplexing Oligos, one for each column of a 96-well plate. .

b. Dispense **105 µl of** Cell Multiplexing Oligo into its appropriate column of a 96-well plate. If a full plate is not necessary, distributing Cell Multiplexing Oligos in a partial plate is acceptable. If using a partial plate, 10x Genomics recommends using 2-12 Cell Multiplexing Oligos or staining using multiples of 12 Cell Multiplexing Oligos (24, 48, etc).

c. Seal plate with a plate sealer and leave at room temperature until use. Plate should be prepared shortly before use.

Cell Multiplexing Oligos in PCR Tubes Preparation

a. Cell Multiplexing Oligos may be prepared in 0.2-ml or 0.5-ml PCR tubes. Ensure that a multichannel pipette can still be used with the chosen tube format.

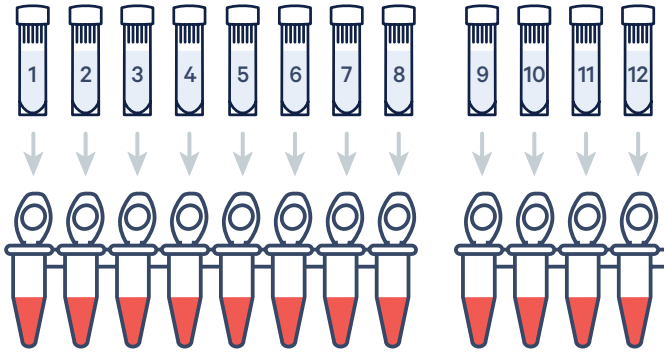


Figure 2. Cell Multiplexing Oligos are distributed into individual tubes.

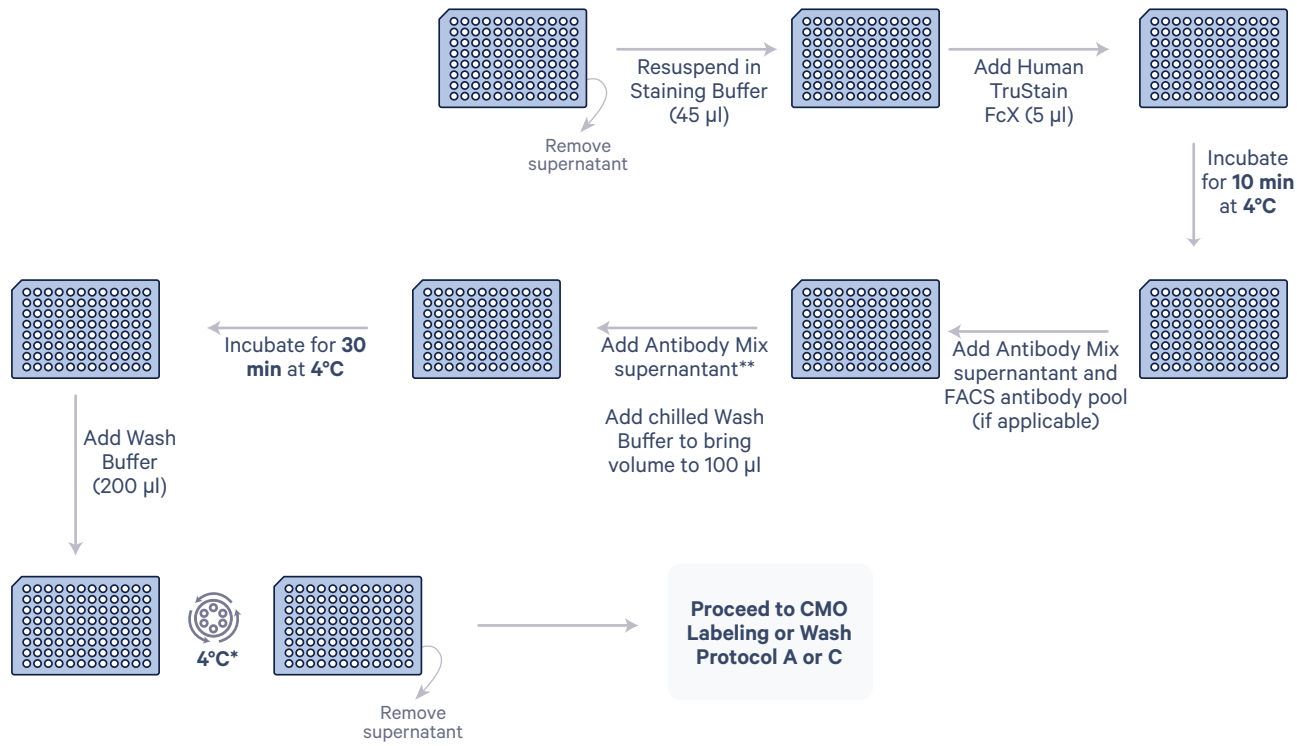
2. Cell Labeling

Overview

This chapter provides guidance on antibody and CMO labeling. If only one labeling protocol is desired, choose either antibody or CMO labeling and proceed to Chapter 3: Cell Washing.

2.1 Antibody Labeling Protocol

Antibody Labeling



*Centrifugation speed and time is sample dependent

**Add FACS antibody pool if applicable

Antibody labeling should always be performed before Cell Multiplexing Oligo labeling. If antibody labeling is not desired, proceed to step 2.2. If only antibody labeling is preferred, proceed to Wash Protocol A after the antibody labeling protocol. FACS may be performed if sample enrichment and/or clean up is preferred. All steps should be performed in a 96-well plate

Antibody labeling is only supported for optimal titrated antibody pools.

Preparing Antibody Mix Supernatant

- Titrate antibodies of interest and perform appropriate quality control before starting this protocol. Do NOT use untitrated antibodies with this protocol. Consult Technical Note Quality Control of Cell Surface Protein Labeling using Flow Cytometry (CG000231) for more information.
- If using a custom lyophillized antibody: Resuspend the antibody-oligonucleotide conjugates in an appropriate volume of staining buffer.
- Centrifuge the mix at **14,000 rcf** for **10 min** at **4°C** to prevent antibody aggregates.
- 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.

Wash Buffer should be chilled PBS + 1% BSA for less fragile cells, chilled PBS + 10% FBS for fragile cells or alternate appropriate staining buffer. Serum concentrations should be at least 1% BSA or 2% FBS to block non-specific binding. Refer to Table 2 on page 5.

Protocol Steps

a. Remove the supernatant without disturbing the pellet. A multichannel vacuum pump is recommended.

TIPS When using the vacuum pump, place the 96-well plate at a 45° angle and touch the side walls with the pipette tip DO NOT touch the pellet. **Remove the entire supernatant.** This guidance applies to all wash steps.

b. Resuspend cell pellet in **45 µl** chilled PBS + 1% BSA or Staining Buffer.

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

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

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

f. Add chilled Wash Buffer to the cells to bring the total volume to **100 µl**. Gently pipette mix 10x (pipette set to 90 µl).

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

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

h. Quench by adding **200 µl** chilled Wash Buffer to the cells. Gently pipette mix.

i. Centrifuge at **4ºC**. Centrifugation speed and time depends upon the sample type. Use Table 1 for guidance.

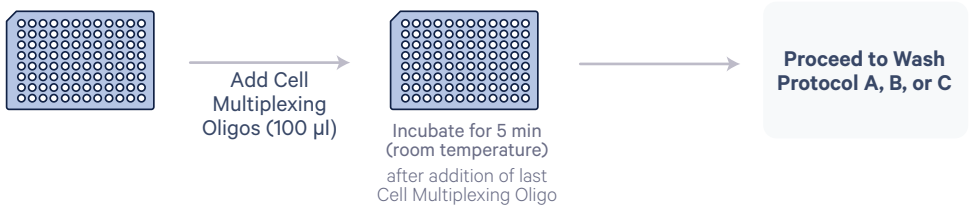
j. Remove the supernatant without touching plate bottom to avoid dislodging the pellet.

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

k. Proceed to High Throughput Cell Multiplexing Oligo labeling or directly to Wash Protocol A or C if Cell Multiplexing Oligo labeling is not desired.

2.2 Cell Multiplexing Oligo Labeling Protocol

Cell Multiplexing Oligo Labeling



*Centrifugation speed and time is sample dependent

Refer to step 1.3 for Cell Multiplexing Oligo preparation instructions.

Protocol Steps

a. Using a multichannel pipette, add **100 µl** Cell Multiplexing Oligo from the prepared plate, tube strip, or 0.5 ml tubes (**room temperature**) to the samples. Immediately and gently pipette mix 10 – 15x to resuspend after each Cell Multiplexing Oligo addition.

b. Incubate for **5 min** at **room temperature** upon completion of Cell Multiplexing Oligo addition.

c. Proceed to appropriate washing protocol.

3. Cell Washing

3.1 Wash Protocols

Refer to page 6 on choosing a wash protocol. 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. Things to consider for each wash are described below:

Wash Protocol A: Plate-based Wash - *page 14*

- This option is appropriate if a plate-based wash method is desired and the sample requires minimal cleanup.

Wash Protocol B: Tube-based Wash - *page 15*

- This option is appropriate if a plate-based wash method is not desired. Large volume washes may help with sample clean up, if large amounts of debris are a concern. Large volume washes can reduce background noise, but may lead to increased multiplet rates.

Wash Protocol C: Tube-based Wash with FACS* - *page 16*

- This option is appropriate for very fragile cells that may have low starting viability and may not survive multiple centrifugation steps. This option also may be used for any cell type if sample clean up via FACS is desired.

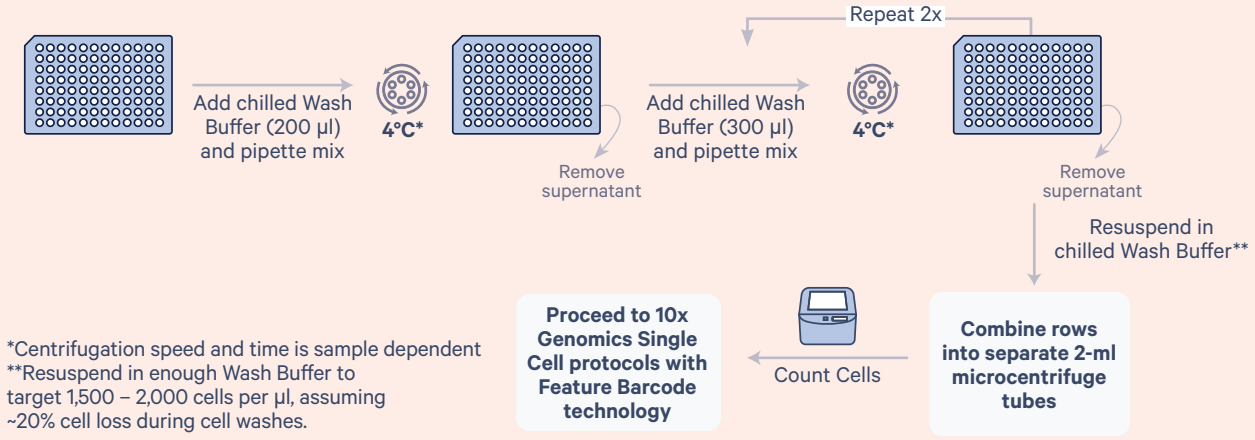
Table 3: Wash Protocol Summary

	Wash Protocol A	Wash Protocol B	Wash Protocol C*
Labeling/Wash Buffer (Chilled 4°C)	Less fragile cells: PBS + 1% BSA Fragile cells: PBS + 10% FBS *(or alternate optimal media)*	PBS + 10% FBS PBS + 1% BSA *(or alternate optimal media)*	PBS + 10% FBS PBS + 1% BSA *(or alternate optimal media)*
Centrifugation Condition**	300-400 rcf, 5 min 4°C	300 rcf, 5 min 4°C	300 rcf, 5 min 4°C
AB Labeling Compatible	✓	✓	✓
CMO Staining Compatible	✓	✓	✓
Wash 1, 2, 3	✓	⊘	⊘
Pool	✓	✓	✓
Pooled Wash 1	⊘	✓	✓
Pooled Wash 2, 3, 4	⊘	✓	⊘
FACS	⊘	⊘	✓

*Wash Protocol C can be used on any sample type if FACS enrichment for a certain cell population or FACS to clean debris is desired.
**Centrifugation conditions may need optimization based on cell type.

3.2 Wash Protocol A: Plate-based Wash

Wash Protocol A



Protocol Steps

a. Add **200 µl** chilled Wash Buffer to the cells to quench the reaction. Gently pipette mix and centrifuge at **4°C.**

b. Remove supernatant with a multichannel vacuum pump without disturbing the pellet.

TIPS

When using the vacuum pump, place the 96-well plate at a 45° angle and touch the side walls with the pipette tip DO NOT touch the pellet. **Remove the entire supernatant.** This guidance applies to all wash steps.

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

c. Add **300 µl** chilled Wash Buffer to the cells. Gently pipette mix and centrifuge at **4°C.**

d. Remove supernatant without touching bottom of plate to avoid dislodging the pellet.

e. **Repeat** c-d for a total of two washes.

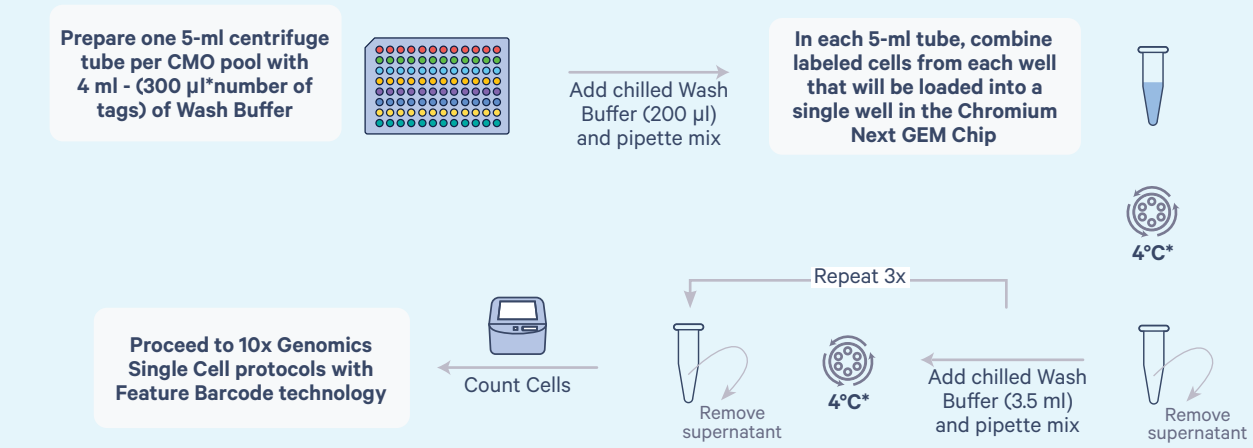
f. Based on starting concentration, add an appropriate volume chilled Wash Buffer.

g. Combine* each row into a separate 2-ml microcentrifuge tube. Each combined sample should contains different oligos. Filter pooled sample with a 30-40 **µm filter to avoid chip clogs.**

- ***Pool samples based on starting cell counts. If cell counts between samples are the same, pool equal volumes. If cell counts are not the same, pooling unequal volumes is acceptable. The 3' CellPlex assay supports sample mixing from 50:50 to 5:95 ratios, although optimal multiplet detection occurs when samples are mixed in equal ratios.
- Determine cell concentration and viability using an automated cell counter such as a Countess II/III or Cellaca MX High-throughput Automated Cell Counter or a hemocytometer and proceed immediately to relevant Chromium Single Cell RNA Sequencing protocols with Feature Barcode technology (see References).

3.3 Wash Protocol B: Tube-based Wash

Wash Protocol B



*Centrifugation speed and time is sample dependent

Protocol Steps

a. For each desired Cell Multiplexing Oligo pool, prepare a 5-ml centrifuge tube with **4 ml – (300 µl * Number of Tags)** of Wash Buffer.

*Example: If performing a 6 Cell Multiplexing Oligo experiment, 4 ml – (6*300 µl) = 2.2 ml of chilled buffer should be added to the 5-ml centrifuge tube.*

b. Add **200 µl** Wash Buffer to the cells. Gently pipette mix

c. In the prepared 5-ml centrifuge tube or tubes, combine labeled cells from each well that will be loaded into a single well on the Chromium Next GEM Chip.

d. Centrifuge at **4°C** . Centrifugation speed and time depends upon the sample type. Use Table 1 for guidance.

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

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

f. Add **3.5 ml** Wash Buffer to the cells. Gently pipette mix and centrifuge at **4°C.**

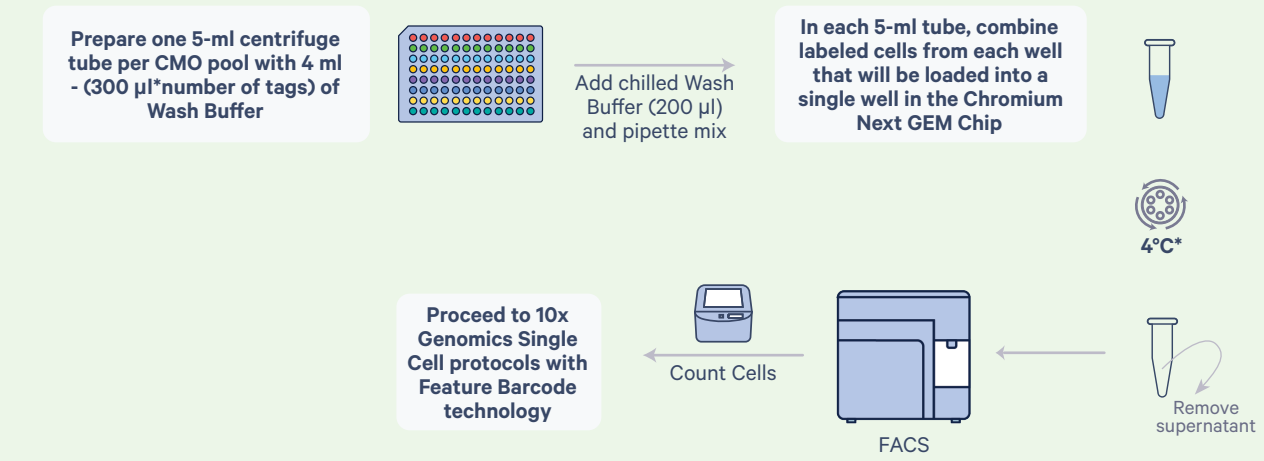
g. Remove supernatant without touching the bottom of the tube to avoid dislodging the pellet.

h. **Repeat** f - g 3x for a total of four washes.

i. Determine cell concentration and viability using an automated cell counter such as a Countess II/III or Cellaca MX High-throughput Automated Cell Counter or a hemocytometer and proceed immediately to relevant Chromium Single Cell RNA Sequencing protocols with Feature Barcode technology (see References).

3.4 Wash Protocol C: Tube-based Wash with FACS

Wash Protocol C



*Centrifugation speed and time is sample dependent

Protocol Steps

a. For each desired Cell Multiplexing Oligo pool, prepare a 5-ml centrifuge tube with **4 ml – (300 µl * Number of Tags)** of chilled PBS + 1% BSA (non-fragile cells) or chilled PBS + 10% FBS (fragile cells).

*Example: If performing a 6 Cell Multiplexing Oligo experiment, 4 ml – (6*300 µl) = 2.2 ml of chilled buffer should be added to the 5-ml centrifuge tube.*

b. Add **200 µl** Wash Buffer to the cells. Gently pipette mix.

c. In the prepared 5-ml centrifuge tube or tubes, combine labeled cells from each well that will be loaded into a single well on the Chromium Next GEM Chip.

d. Centrifuge at **4°C** . Centrifugation speed and time depends upon the sample type. Use Table 1 for guidance.

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

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

f. For enrichment of labeled cells by FACS:

- Based on starting concentration and assuming up to ~50% cell loss, add an appropriate volume chilled PBS + 10% FBS or PBS + 1% BSA (including a dead cell marker) to obtain a final cell concentration of 5-10 x 10^6 cells/ml and proceed to FACS.
- Only one sample pool can be sorted at a time; thus, the remaining samples may remain on ice for an extended period at a time. Extensive wait times should be avoided by loading cells as soon as possible into 10x Genomics chips.

- DNA-based dead cell markers should be added to cell suspension **1-5 min** before sorting.
- Sample tube and collection chamber should remain at **4ºC**.
- Collection buffer should be optimal for the sample and should contain a higher than normal concentration of serum or BSA, as FACS will dilute the collection buffer as cells are sorted. The same guidance applies for BSA.
- Collection buffer volume will depend on the chosen flow sorter, nozzle, sort mode, and desired number of sorted cells.

  Example: A Sony MA900 sorter on purity collection mode with a 100 µm nozzle will result in around 3 nl of volume per collection event. 100,000 sorted cells will result in 300 µl of collected FACS sheath buffer. Loading 100 µl of collection buffer in the tube prior to sorting at 4% BSA will result in a more appropriate 1% BSA concentration after sorting.

- Ensure that the collection tube is pre-coated with collection buffer.
- Adjust FSC and SSC/BSC voltage, excluding doublets and dead cells.
- After FACS, centrifuge cells, remove supernatant, and resuspend in appropriate volume assuming up to ~50% cell loss. Centrifugation for longer times may result in better cell recovery.
- Determine cell concentration and viability using an automated cell counter such as a Countess II/III or Cellaca MX High-throughput Automated Cell Counter or a hemocytometer and proceed immediately to relevant Chromium Single Cell RNA Sequencing protocols with Feature Barcode technology (see References).

Appendix

Sample Quality Improvements for Pooled Samples
High quality samples should maximize cell viability and minimize debris and cell aggregates. If necessary, clean up the sample by:

- Flow sorting for viable cells
- Centrifuge at low speed to remove debris
- Sample filtration

For more information, consult the Best Practices to Minimize Chromium Next GEM Chip Clogs and Wetting Failures Technical Note (CG000479).

References

The protocol outlines labeling cells with Antibodies and Cell Multiplexing Oligos for use with:

1. Chromium Next GEM Single Cell 3' Reagent Kits v3.1 (Dual Index) with Feature Barcode technology for Cell Multiplexing User Guide (CG000388)
2. Chromium Next GEM Single Cell 3' Reagent Kits v3.1 (Dual Index) with Feature Barcode technology for CRISPR Screening and Cell Multiplexing User Guide (CG000389)
3. Chromium Next GEM Single Cell 3' Reagent Kits v3.1 User Guide with Feature Barcode technology for Cell Surface Protein and Cell Multiplexing (CG000390)
4. Chromium Next GEM Single Cell 3' HT Reagent Kits v3.1 User Guide with Feature Barcode technology for Cell Multiplexing (CG000419)
5. Chromium Next GEM Single Cell 3' HT Reagent Kits v3.1 User Guide with Feature Barcode technology for Cell Surface Protein and Cell Multiplexing (CG000420)
6. Chromium Next GEM Single Cell 3' HT Reagent Kits v3.1 User Guide with Feature Barcode technology for CRISPR Screening and Cell Multiplexing (CG000421)
7. 10x Genomics Single Cell Protocols Cell Preparation Guide (CG000053)
8. Guidelines for Accurate Target Cell Counts using 10x Genomics Single Cell Solutions (CG000091)
9. Thawing Dissociated Tumor Cells for Single Cell RNA Sequencing (CG000233)
10. Fresh Frozen Humaner Peripheral Blood Mononuclear Cells for Single Cell RNA Sequencing (CG000039)
11. Best Practices to Minimize Chromium Next GEM Chip Clogs and Wetting Failures Technical Note (CG000479).
12. Quality Control of Cell Surface Protein Labeling using Flow Cytometry (CG000231).
13. Cell Multiplexing Oligo Labeling for Single Cell RNA Sequencing Protocols with Feature Barcode Technology (CG000391).

Document Revision Summary

Document Number	CG000426
Title	Plate-based Sample Preparation for Single Cell RNA Sequencing
Revision	Rev C
Revision Date	April 2022

Specific Changes:

- Updated title to "Plate-based Sample Preparation for Single Cell RNA Sequencing
- Updated introduction to reflect addition of antibody labeling protocol
- Added Table of Contents
- Added vacuum trap system (Thermo), vacuum pump (Fisher), polystyrene test tubes (Fisher), BSA (Miltenyi), and LoBind tubes (Eppendorf) to Specific Reagents and Consumables table
- Added General Protocol Overview to reflect additional sections (page 3)
- Added Tips & Best Practices section (pages 4-6)
- Added information on preparing CMOs in PCR tubes (page 9)
- Added antibody labeling protocol (pages 10-12)
- Added multiple wash protocols (page 13-17)
- Updated references to include CG000390, CG000421, CG000053, CG000091, CG000233, CG000039, CG000231, and CG000391

General Changes:

- Updated for general minor consistency of language and terms throughout

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