User Manual

TotalSeq™ PhenoCyte 200k Reagent Kits

For use with:

TotalSeq™-A PhenoCyte Human Universal Kit, V1.0 (200k)
Cat#399911

LBL-03162 [01]

BioLegend®

It is highly recommended that this manual be read in its entirety before using this product. Do not use this kit beyond the expiration date.

For Research Purposes Only. Not for use in diagnostic or therapeutic procedures. Purchase does not include or carry the right to resell or transfer this product either as a stand-alone product or as a component of another product. Any use of this product other than the permitted use without the express written authorization of BioLegend is strictly prohibited.

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Introduction and General Information

Introduction

The TotalSeq™-A PhenoCyte Human Universal Kit, V1.0 provides an end-to-end solution for high-throughput, high-parameter single-cell protein analysis. This kit combines BioLegend's pre-titrated, optimized TotalSeq™-A antibody cocktails with Scale Bio™ Quantum Barcoding technology to enable precise multiplexing of hundreds of protein markers using viable single-cell suspensions as the primary input. Designed for scalability and efficiency, the kit provides a streamlined, instrument-free workflow to obtain high-sensitivity, low-background data: capable of detecting rare cell populations with frequencies as low as 1 in 10,000 cells. The data generated is processed and analyzed using the free cloud-based Multiomics Analysis Software (MAS). This software provides a wide range of analysis tools, including traditional flow-like bivariate gating and protein clustering for phenotyping populations. Additionally, it offers advanced dimensionality reduction capabilities and an interactive plot interface for creating publication-quality images and figures.

Workflow Overview

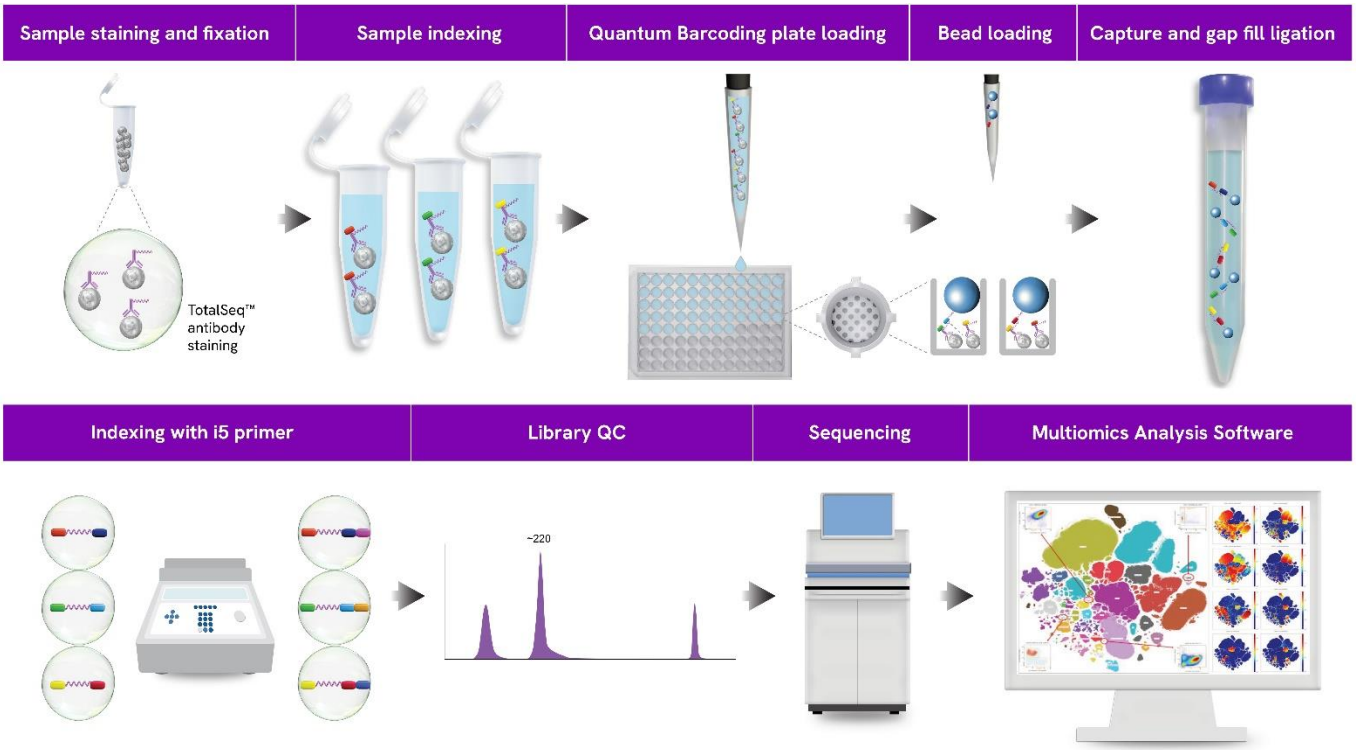


Figure 1. *Step-wise overview of the TotalSeq™ PhenoCyte workflow.*

Cells are initially stained with TotalSeq™ antibodies and subsequently fixed with paraformaldehyde. Following fixation, the sample(s) are indexed with Sample Indexing Oligos (i7 primers) which hybridize to the antibody oligos. The indexed cells are loaded onto the Quantum Barcoding plate that partitions cells into microwells. Quantum Barcoding Protein Beads containing the second index (bead barcode) are then loaded onto the plate and hybridize directly to the poly(A) tail of the antibody oligos. Post-hybridization, the beads now containing the hybridized sequences are collected and undergo a gap-fill ligation to bridge the indexing sequences on the antibody oligo. The ligated product undergoes a final round of indexing via i5 indexing PCR and the library is amplified. A final library consists of combinations of unique identifiers: the Sample Index, the antibody barcode, the bead barcode, and the i5 index. The identifiers help facilitate the sequencing of the libraries and the

identification of individual cells in the downstream analysis. Following library QC and sequencing the data can be processed and analyzed with the Multiomics Analysis Software.

Protocol Workflow and Stopping Points

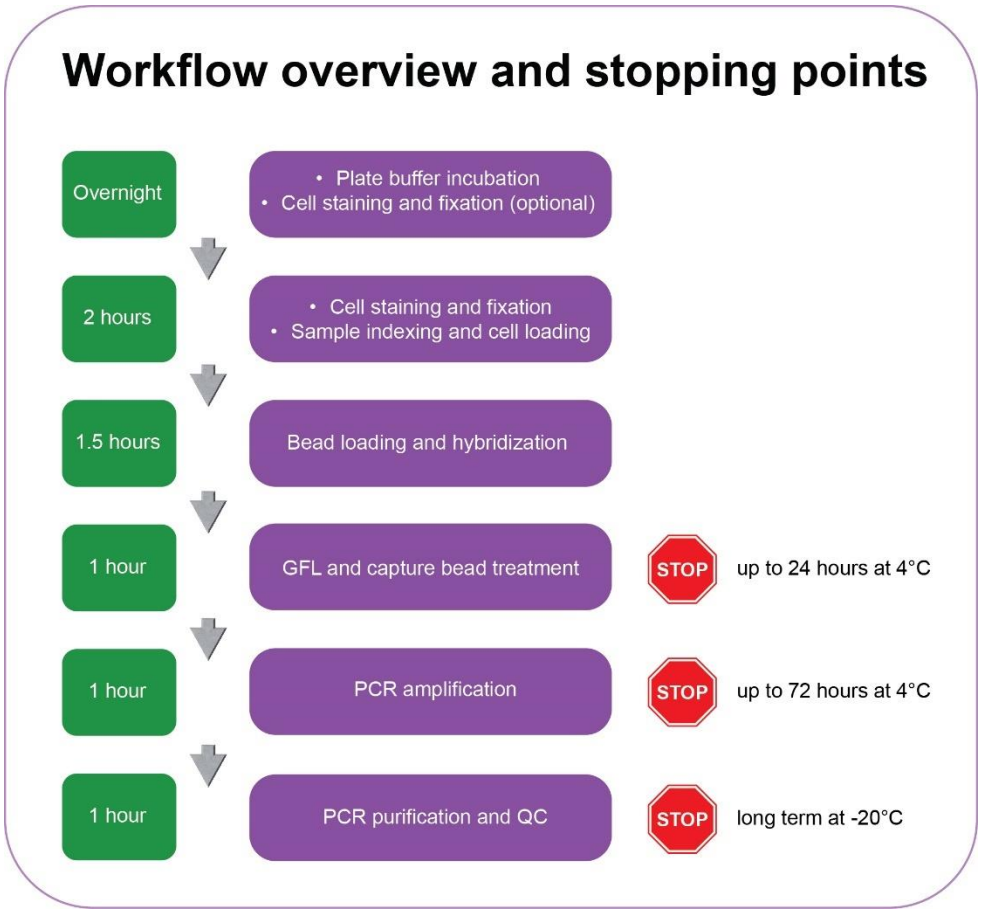


Figure 2. Workflow overview and protocol stopping points.

Note: The plate buffer incubation requires a minimum of 16 hours or a maximum of 72 hours so it is recommended to be performed overnight (Day 0). Cell staining and fixation can be performed on either Day 0 or Day 1 depending on the experimental setup. For Day 0, store fixed cells in Cell Staining buffer until ready for use (2-8°C for 24hrs max).

Tips and Best Practices

General laboratory best practices:

- Calibrate and service pipettes every 12 months to ensure accurate sample volume transfer at each step.
- Store all reagents at the storage conditions recommended by the supplier.
- Unless otherwise specified, thaw reagents on ice.
- Never reuse pipette tips or tubes.
- Keep pipette tip boxes, reagent containers, and sample tubes closed when not in use.
- Wear suitable protective clothing, eyewear and gloves.

For prevention of amplicon cross-contamination in sequencing libraries:

- Thaw and prepare reagent mixes in pre-amplification workspaces.

- Perform amplification in post-amplification workspaces.
- Perform PCR purification steps in a post-amplification workspace
- Never bring material or equipment from post-amplification workspaces into pre-amplification workspaces.
- Regularly clean post-amplification workspaces with a 10% bleach solution.

Scale Bio™ Quantum Barcoding and Library Structure

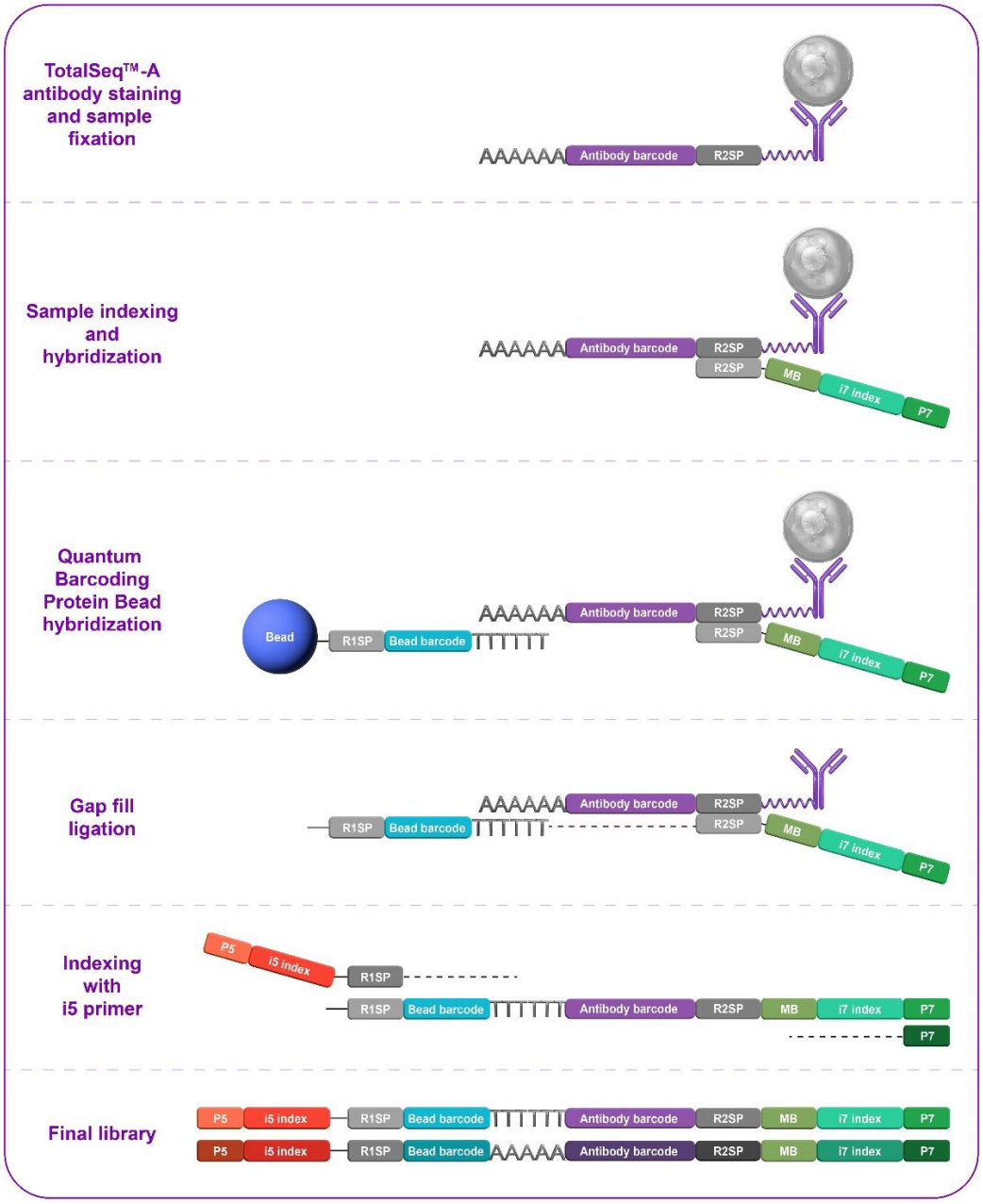


Figure 3. Quantum Barcoding and library construct workflow.

Note: A detailed stepwise overview of the library structuring is provided in the Appendix: Quantum Barcoding and Indexing Sequences.

Kit Components and Third-Party Reagents/Instruments

TotalSeq™ PhenoCyte Kit Components

Kit Components	Part Number	Qty	Storage Condition
TotalSeq™-A PhenoCyte Human Universal Kit, V1.0	***399911***	***1***	***Various***
TotalSeq™-A PhenoCyte - Module A	750004373	1	-30°C to -10°C
TotalSeq™-A PhenoCyte - Module B	750004374	1	-30°C to -10°C
TotalSeq™-A PhenoCyte - Module C: Human Universal, V1.0	750004375	1	2°C to 8°C

Module A Components	Part Number	Qty	Storage Condition
TotalSeq™-A PhenoCyte - Module A	***750004373***	***1***	***-30°C to -10°C***
Sample Indexing Oligos	750004351	1	-30°C to -10°C

Module B Components	Part Number	Qty	Storage Condition
TotalSeq™-A PhenoCyte - Module B	***750004374***	***1***	***-30°C to -10°C***
GFL Additive	750004336	1	-30°C to -10°C
GFL Buffer A	750004338	1	-30°C to -10°C
GFL Buffer B	750004340	1	-30°C to -10°C
GFL Enzyme A	750004342	1	-30°C to -10°C
GFL Enzyme B	750004344	1	-30°C to -10°C
PCR Master Mix	750004346	1	-30°C to -10°C
Library Oligos	750004348	1	-30°C to -10°C

Module C Components	Part Number	Qty	Storage Condition
TotalSeq™-A PhenoCyte - Module C: Human Universal, V1.0	***750004375***	***1***	***2°C to 8°C***
Quantum Barcoding Protein Beads	750004349	1	2°C to 8°C
Wetting Buffer	750004352	1	2°C to 8°C
Hyb Buffer A	750004354	1	2°C to 8°C
Purification Buffer	750004356	1	2°C to 8°C
Hyb Buffer B	750004358	1	2°C to 8°C
Bead Collection Buffer	750004360	1	2°C to 8°C
Treatment Solution	750004362	1	2°C to 8°C
Sample Collection Funnel	750004365	1	2°C to 8°C or RT
Quantum Barcoding Plate	750004366	1	2°C to 8°C or RT
TotalSeq™-A Human Universal Cocktail, V1.0 (x1 Rxn)	750004380	1	2°C to 8°C

Supplemental Reagents Required

Additional reagents needed for Cell Surface Staining and Fixation:

Consumable	Vendor	Part Number
Cell Staining Buffer	BioLegend	420201
Human TruStain FcX™	BioLegend	422301
1x PBS	BioLegend	926201 or equivalent
Fixation Buffer	BioLegend	420801
Low Protein Binding Microcentrifuge Tubes	Fisher Scientific	Cat. No. 90410 or equivalent
12 x 75 mm Falcon™ Round-Bottom Polystyrene Tubes	Fisher Scientific	Cat. No. 14-959-1A or equivalent

Additional reagents needed for Quantum Barcoding, PCR, & Library Quantification:

Consumable	Vendor	Part Number	Qty Needed
Nuclease Free Water	Various	Various	5 mL
Elution Buffer (10mM Tris, pH 8.0) or equivalent	Various	Various	50 µL
Adhesive Plate seals	Various	Various	3 Seals
Sterile, filtered, low retention tips for P1000, P200, and P20 pipettes	Various	Various	1 boxes of P1000
			5 boxes of P200
			2 boxes of P20
Sterile, filtered, wide bore tips for P1000 and P200 pipettes	Various	Various	1 box of P1000
			1 box of P200
0.2-mL PCR 8-strips	Axygen	PCR-0208-CP-C	2 8-strips (optional)
Or			Or
96-well PCR Plates			2 Plates
1.5-mL LoBind Eppendorf tubes	Eppendorf	30108418	5 Tubes
2.0-mL LoBind Eppendorf tubes	Eppendorf	30108310	1 Tube
15-mL Tube	Various	Various	1 Tube
Cell counting dye: AO/PI, Trypan Blue, YOYO-1, etc.	Various	Various	1 bottle
SPRIselect Size Selection Beads	Beckman Coulter	B23317	1 mL
Ethyl Alcohol	Various	Various	3 mL
Qubit dsDNA HS Assay Kit	ThermoFisher	Q32851, Q32854, Q33230, Q33231	1 kit
D5000 ScreenTape Kit or equivalent	Agilent	5067-5588	1 kit
Reagent Reservoir	Various	Various	Multiple

Equipment and Instrument List

Consumable	Vendor	Part Number
Centrifuges for 8-strip, 1.5-mL tubes, and 96-well plates	Various	Various
Vortex mixer, thermomixer, or MixMate	Various	Various
P1000, P200, P20, P2 pipettes	Various	Various
P200, P20, P10 multichannel pipettes*	Various	Various
Thermocycler	Various	Various
96-well plate magnet	Various	Various
1.5/2.0-mL tube magnet	Various	Various
15-mL magnetic stand	Various	Various
Hula/Rotator/Orbital Mixer	Various	Various
Qubit 4 Fluorometer	Thermo Fisher	Q33238
4200 TapeStation Instrument or equivalent DNA trace analyzer	Agilent	G2991BA
Illumina Sequencer (see sequencing requirements)	Illumina	Various

Note: * It is strongly recommended to use multichannel pipettes throughout the protocol due to the volume of aliquoting and use of 96 well plates.

Step 1: Preparing the Quantum Barcoding Plate

- Plate Buffer Incubation
- Cell Staining Planning

Step 1: Preparing the Quantum Barcoding Plate

The Quantum Barcoding Plate requires incubation with the supplied wetting buffer for a minimum of 16 hours or a maximum of 72 hours. This step is recommended to be performed overnight before proceeding to any plate loading.

Before you begin: Set a swing bucket centrifuge to room temperature with adaptors that can hold 96-well plates.

Please review the table below to prepare reagents before starting this protocol section:

Source	Consumable	Storage Temp	Place
TotalSeq™-A PhenoCyte - Module C	Quantum Barcoding Plate	2°C to 8°C or RT	RT
	Wetting Buffer	2°C to 8°C	Ice
	Hyb Buffer A	2°C to 8°C	Ice
Other vendors	Adhesive Plate Seals	-	-

Caution: when pipetting from the Quantum Barcoding Plate, always remove liquid from a selected corner of the wells so as not damage them, an example is shown below. Ensure the same corner is used every time for all pipetting steps.

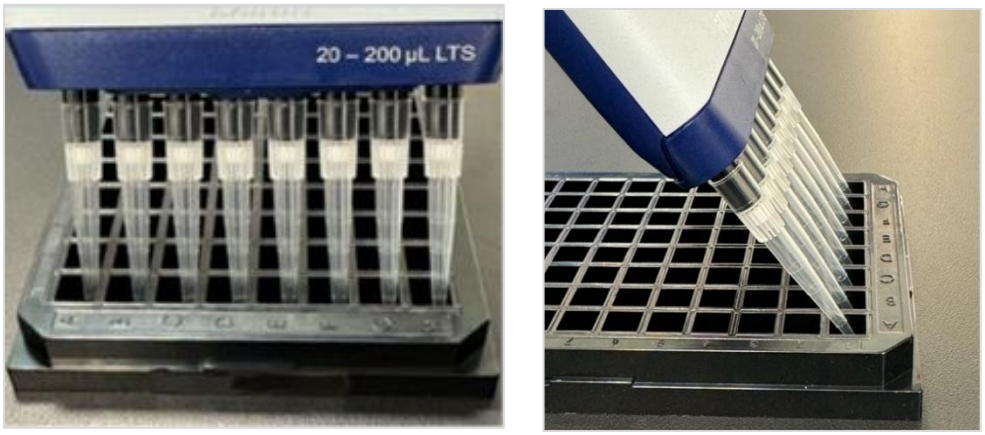


Figure 4. The described method of pipetting out of the Quantum Barcoding Plate wells. Always ensure the liquid is removed from the same corner of a well.

Plate Buffer Incubation

Procedure:

1. Open the packaging of the Quantum Barcoding Plate. Set aside the plate lid on a clean surface; the lid will be used throughout the protocol.
2. Using a multichannel pipette, add 100 µl of Wetting Buffer to ***16 wells*** (the first two columns of the plate) by dispensing the liquid on the side walls. Seal the plate with an adhesive plate seal.
   Note: It is recommended to aliquot Wetting Buffer into a reagent reservoir for use while loading the plate with a multichannel pipette.
3. Place the plate lid on the Quantum Barcoding Plate and spin down at 300 x g for 5 min at RT with an appropriately weighted balance.

4. Incubate at 4°C for a minimum of ***16 hours or a maximum of 72 hours***.
5. Remove the Wetting Buffer from each well by pipetting from a selected corner of the wells as shown in Figure 4.
6. Wash the 16 wells by adding 150 µL of Hyb Buffer A to each well by dispensing liquid along the side of the well.
7. Remove the Hyb Buffer A from the 16 wells by aspirating the liquid from a selected corner of the well as shown in Figure 4.
8. Repeat steps #6-7 two more times for a total of 3 washes.
9. Add 150 µL Hyb Buffer A into each of the 16 wells of the Quantum Barcoding Plate. Place the lid on the plate. Incubate at room temperature for at least one hour or up to a maximum of 24 hours.

Cell Staining Planning

Following the initial 16-72-hour plate buffer incubation on Day 0, users can proceed to Step 2: Cell Staining and Fixation on Day 1 during the second plate incubation at room temperature. Note: In this scenario sample prep, staining/fixation and Step 3: Sample Indexing, would all be performed on the same day (Day 1).

Alternatively, users can perform the antibody staining earlier on Day 0, in the case of sample batching or simplifying protocol timeframes. Following sample staining and fixation, cells can be stored in Cell Staining Buffer (or equivalent) for a ***maximum of 24 hours at 2-8°C***. It is not recommended to use cells that have been stored fixed for an extended period of time since that can cause issues with the data quality. Note: In this scenario, the sample prep and staining/fixation can be done on Day 0 while the plate incubates. Users would then store the cells until they are ready to proceed to Step 3: Sample Indexing the next day (Day 1).

Step 2: Cell Surface Staining and Fixation

- Cell Preparation Guidance
- Sample Multiplexing with TotalSeq™ Hashtags *(Optional)*
- Cell Staining with TotalSeq™ antibody cocktails
- Fixation and Quenching

Step 2: Cell Surface Staining and Fixation

Cell Preparation Guidance

This protocol has been optimized using viable single-cell suspensions of fresh human PBMCs isolated using density gradient centrifugation. If using cells isolated with a different procedure, users may need to verify the antibody staining patterns using alternative methods since they can vary depending on tissue type and viability. It is recommended to use highly viable (>95%) single-cell suspensions with minimal debris and cell aggregates to ensure optimal data quality. BioLegend has not tested this protocol using single-cell suspensions derived from enzymatically digested tissue. Enzymatic digestion may result in alterations of surface protein epitopes and impact staining with TotalSeq™ antibodies. Optimization of sample processing, staining conditions, and concentrations may be required.

Sample Multiplexing with TotalSeq™ Hashtags (Optional)

TotalSeq™-A hashtag reagents are compatible with the TotalSeq™ PhenoCyte kit and can be used to increase sample throughput. If you plan to multiplex samples using TotalSeq™ hashtags, there are two commonly used approaches to staining samples with hashtag antibodies.

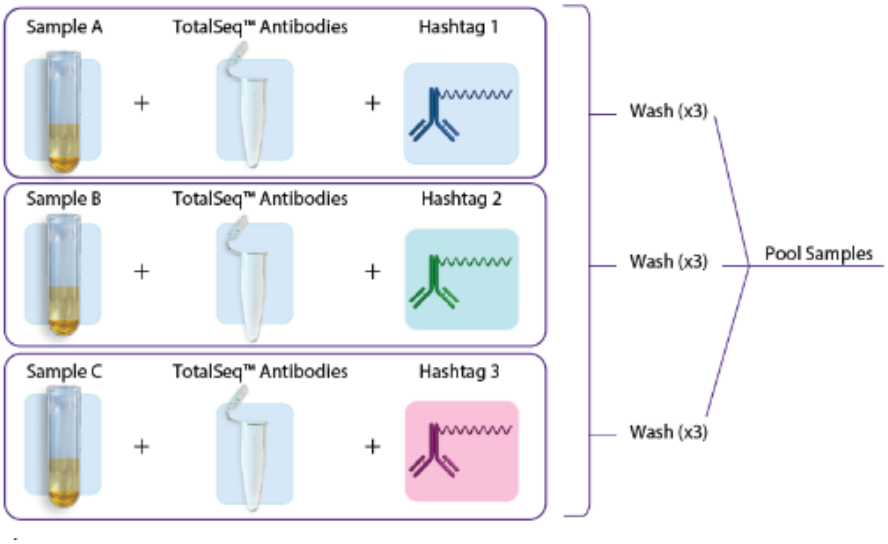


Figure 5. Staining with TotalSeq™ antibodies and hashtags in a single step before pooling samples.

In the first approach, individual samples are stained with pre-optimized TotalSeq™-A cocktails and hashtags in a single step, washed three times to remove unbound antibodies, and subsequently pooled for loading into one or more Quantum Barcoding reactions, See Figure 5. For the TotalSeq™ PhenoCyte kits, this approach may require additional TotalSeq™ cocktails depending on the kit size and number of samples being multiplexed. After staining, proceed to the Fixation and Quenching steps.

Note: Purchase of additional TotalSeq™ cocktails may be required depending on the kit size and specific experimental set up.

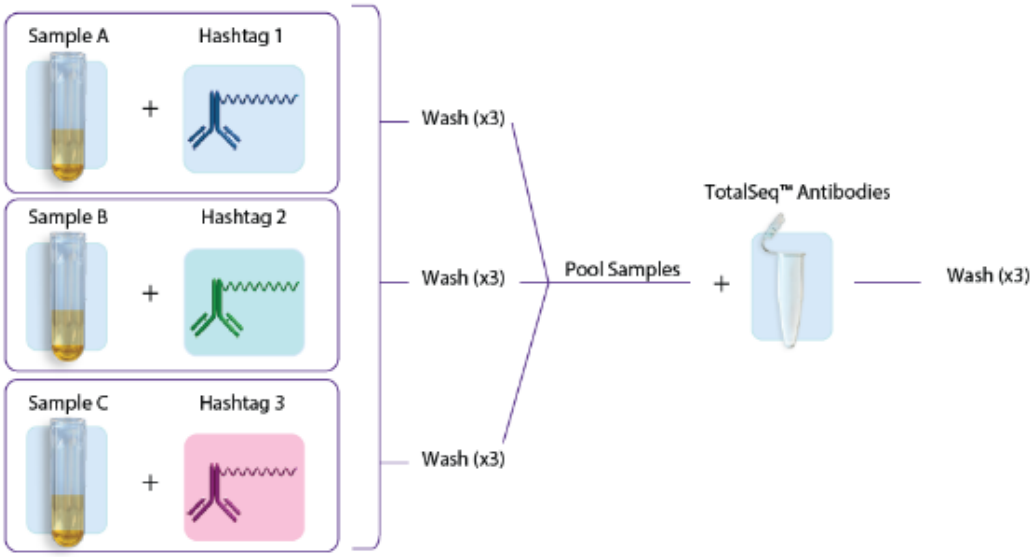


Figure 6. Staining individual samples with hashtag reagents followed by pooling and staining with additional TotalSeq™ antibodies in subsequent steps

The second approach involves first staining individual samples with the hashtags, washing three times, and sample pooling. The pooled samples would then be stained with the TotalSeq™ antibodies followed by three additional washes. See Figure 6. After staining, proceed to the Fixation and Quenching steps.

With either approach, samples are recommended to be pooled equally and the volume adjusted to the appropriate concentration as required by this kit. It is highly recommended to titrate the hashtag reagents prior to use to ensure compatibility with the sample and for adequate read balancing. Using too much hashtag reagent can cause overconsumption of reads leading to read balancing issues with the generated library (e.g. hashtags consuming too many reads leading to poor representation of low/intermediately expressed markers). For any questions regarding use of TotalSeq™ Hashtags with the TotalSeq™ PhenoCyte kits, please reach out to BioLegend's Technical service team for assistance.

Note: For 1.2M size kits, a total minimum of 2.4 x10^6 fixed and stained cells is required for sample indexing. For 200K size kits, a total minimum of 400,000 fixed and stained cells is required for sample indexing. It is not recommended to combine cell hashing with sample index multiplexing (i7 primer).

Cell Staining with TotalSeq™ antibody cocktails

Before you begin:

Ideal cell viability is >95%. Low cell viability is associated with poor single-cell sequencing data. If low viability is observed, users may need to enrich live cells or repeat cell suspension preparation. Note that if hashing is performed, this may affect the following steps (volumes/cell counts/etc.) Please contact BioLegend Technical Services with any questions regarding cell viability or the use of hashtags with this kit.

Please review the table below to prepare reagents before starting this protocol section:

Source	Consumable	Storage Temp	Place
TotalSeq™-A PhenoCyte - Module C	TotalSeq™-A Human Universal Cocktail, V1.0 (1 Rxns)	2°C to 8°C	RT
BioLegend	Cell Staining Buffer	2°C to 8°C	Ice
BioLegend	Human TruStain FcX™	2°C to 8°C	Ice
BioLegend	1x PBS	2°C to 8°C	Ice
BioLegend	Fixation Buffer	2°C to 8°C	Ice
Other Vendors	Low Protein Binding Microcentrifuge Tubes	-	-
Other Vendors	12 x 75 mm Falcon™ Round-Bottom Polystyrene Tubes	-	-
Other Vendors	10x TBS & 1x TBS	-	-

Procedure:

1. Prepare single-cell suspension with the preferred or recommended method.
2. Count and assess cell viability.
    a. Using your preferred method, carefully count cells to ensure accurate quantitation and viability.
3. Dilute $5x10^5$ - $2x10^6$ cells in 22.5 µL of Cell Staining Buffer in a 12 x 75mm flow cytometry tube.
4. Fc receptor blocking.
    a. Add 2.5 µL of Human TruStain FcX Fc blocking reagent to the cells in 22.5 µL of Cell Staining Buffer (Total volume = 25 µL).
    b. Incubate for 10 minutes at 4°C.
    c. While cells are incubating in Fc Block, proceed to the cocktail reconstitution and staining steps.
5. TotalSeq™-A Human Universal Cocktail Reconstitution and Staining.
    a. Equilibrate the lyophilized panel vial to room temperature for 5 minutes.
    b. Place the lyophilized panel vial in an empty microcentrifuge tube, and spin down at 10,000 x g for 30 seconds at room temperature.
    c. Rehydrate the lyophilized panel by adding 27.5 µL of Cell Staining Buffer. Replace the cap and vortex for 10 seconds.
    d. Incubate at room temperature for 5 minutes.
    e. Vortex again and spin down at 10,000 x g for 30 seconds at room temperature.
    f. Transfer the entire volume, 27.5 µL of the TotalSeq™-A Universal Cocktail to a low protein binding microcentrifuge tube.

g. Centrifuge at 14,000 x g for 10 min at 4°C.

Centrifugation of the reconstituted cocktail at 14,000 x g at 2 – 8°C for 10 minutes is critical to ensure the removal of antibody aggregates. It is not recommended to combine multiple cocktails into a single reaction due to the increased risk of aggregates.

h. Transfer 25 µL of the FcR-blocked cells into a 12 x 75mm flow cytometry tubes.

i. Transfer 25 µL of the reconstituted cocktail to the tube containing 25 µL FcR-blocked cells, taking care to avoid the bottom of the tube, for a final staining volume of 50 µL. Mix by gently pipetting 5 times.

j. Incubate for 30 minutes at 4°C.

6. Wash cells.
   a. Add 3 mL of Cell Staining Buffer and mix by gently pipetting 5 times.
   b. Centrifuge at 4°C for 5 minutes at 400 – 600 x *g* depending on your sample type. Decant the supernatant and repeat this steps 6a and 6b total of 3 washes.

It is important to thoroughly decant the wash buffer and resuspend the cell pellet by either pipetting or a gentle vortex. Discard supernatant with a single firm, but not overly forceful motion. Proceed to absorb any remaining liquid on the lip of the tube with a clean paper towel.

c. After the final wash, decant the supernatant and resuspend cells in 200 µL of Cell Staining Buffer for an approximate final volume of 250 – 350 µL. Gently mix the cells by pipetting.

d. Slowly filter cells through 40 µm Flowmi™ Cell Strainer into a low protein binding microcentrifuge tube.

e. Count cells and record cell viability. Proceed immediately to Fixation.

Fixation and Quenching

Procedure:

1. Fixation
   a. Prepare Fixation solution by combining 300uL Fixation Buffer and 900uL PBS 1X.
   b. Add 1mL of Fixation Solution to the tube containing stained cells. Vortex briefly and incubate at room temperature for 20 min.
2. Quenching
   a. Add 100uL TBS 10X. Vortex briefly and incubate at room temperature for 5 min
   b. Add 3mL TBS. Centrifuge at 850g for 5 minutes at room temperature.
   c. Decant supernatant and resuspend cells in the residual volume.
   d. Using your preferred method, carefully count cells to ensure accurate quantitation and record counts.
   e. Keep cells on ice and proceed to Step 3: Sample Indexing and Cell Loading.

Note: Stained and fixed cells can be immediately used for Sample Indexing. Alternatively, fixed samples can be stored in Cell Staining Buffer (or equivalent) for a maximum of 24 hours at 2-8°C. It is not recommended to use cells that have been stored fixed for an extended period since that can cause issues with the data quality.

Step 3: Sample Indexing and Cell Loading

- Sample Indexing
- Quantum Barcode Plate: Cell Loading

Step 3: Sample Indexing and Cell Loading

Before you begin:

- ***Keep fixed and stained cells on ice or 4°C until use.***
  - ***Ensure a total minimum of 400,000 fixed and stained cells are sample indexed. 320,000 of the cells will be loaded into the plate after sample indexing.***
  - ***A minimum of 20,000 fixed and stained cells are required for each index. .***
- ***Set a swing bucket centrifuge to room temperature with adaptors that can hold 96-well plates.***

Note: If samples were hashed, make sure to pool samples before proceeding with Sample Indexing step, depending on the hashing method used (See Sample Multiplexing with TotalSeq™ Hashtags). It is not recommended to combine hashtag multiplexing with sample index (i7) multiplexing.

Please review the table below to prepare reagents before starting this protocol section:

Source	Consumable	Storage Temp	Place
TotalSeq™-A PhenoCyte - Module A	Sample Indexing Oligos	-30°C to -10°C	Ice
TotalSeq™-A PhenoCyte - Module C	Hyb Buffer A	2°C to 8°C	Ice
-	Fixed, Stained Cells	2°C to 8°C	Ice
Other vendors	0.2-mL PCR 8-strips or 96-well plate	-	-

Sample Indexing

Procedure:

1. Centrifuge the fixed, stained cells at ***850 x g for 5 min*** at RT.
2. Decant the supernatant.
3. Add 3 mL of Hyb Buffer A and spin down at ***850 x g for 5 min*** at RT.
4. Decant the supernatant.
5. Resuspend the tube containing cells with 480 µL of Hyb Buffer A.
6. Aliquot 30 µL of the antibody-stained cells into two columns (16-wells) of a 96-well plate. Alternatively, this can be done using two 0.2-mL 8-tube PCR tube strips, ensuring the volume is 30µL per well/tube.
7. Gently vortex or pipette mix the Sample Indexing Oligos and spin down at 100 x g for 1 min.
8. Add 5 µL of a unique Sample Indexing Oligo into each well or tube containing the fixed cells. All 16 i7 indices need to be used so that each well/tube contains a unique index. See ***Sample Indexing Oligos Sequences (for i7 indices)*** table on Page 38.
9. Gently vortex and briefly spin down to ensure the liquid is at the bottom of the tubes or plate wells.
10. Incubate the cell and oligo mixture at 37°C for 45 mins.
    a. If utilizing a thermocycler, set the lid to 45°C.
11. After the incubation is complete, add 100 µL of Hyb Buffer A to each tube/well.

    Note: If using a multichannel pipette, it is recommended to aliquot Hyb Buffer A into a reagent reservoir for use throughout this section of the protocol.
12. Centrifuge the mixture at ***850 x g for 3 min*** at RT.
    a. Use a PCR plate adaptor with a swing bucket centrifuge.

13. Carefully remove 120 µL of supernatant from each well, pipetting from the opposite wall of the expected cell pellet.
    a. Caution: cell pellet may not be visible.
    b. To avoid cell loss, ***slowly*** aspirate from the top of the supernatant with the pipette along the sidewall of the tube on the opposite side from where the cell pellet should be (shown below in).

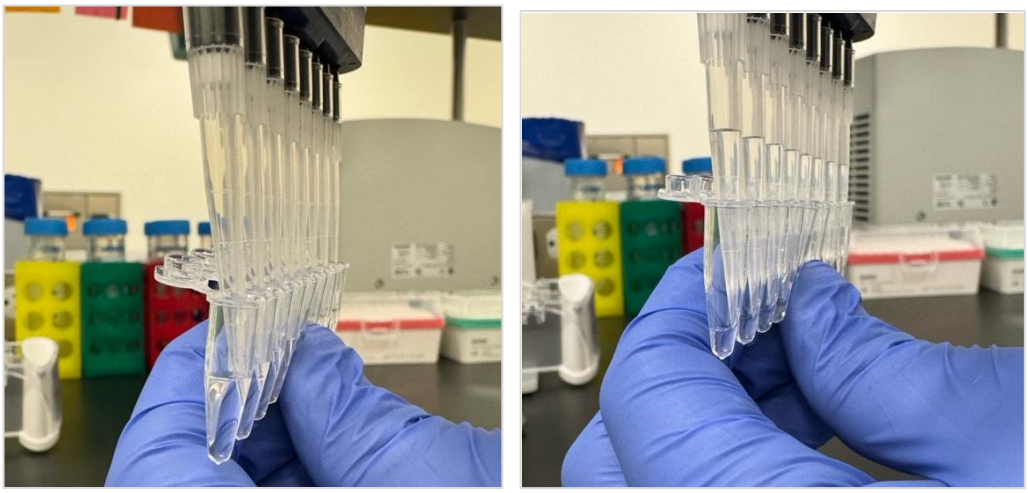


Figure 7. When removing the supernatant from the tubes, carefully pipette along the sidewall of the tube on the opposite side from where the cell pellet is. This is a critical step.

14. Add 100 µL Hyb Buffer A to each tube/well.
15. Centrifuge at ***850 x g for 3 min*** at RT.
16. Carefully remove 100 µL of supernatant from each tube.
17. Add 50 µL of Hyb Buffer A to each tube and resuspend the cell pellet by pipette mixing.
18. Collect and pool all cells and/or samples into a 2-mL or FACS tube.
    a. Ensure each tube is gently pipette mixed before aspirating cells for pooling.
    b. Ensure all samples are combined at this step.
19. Centrifuge at ***850 x g for 3 min*** at RT and decant the supernatant.
20. Add 200 µL Hyb Buffer A to the tube and resuspend the cell pellet by pipette mixing.
21. Determine the concentration of the cell suspension using a cell staining dye and cell counting equipment.

    For accurate cell counting, use ≥ 2 µL of well-mixed cell suspensions and appropriate dilution factors to obtain cell counts that are recommended for accuracy for your cell counting method. Perform cell counts in duplicate, adding additional cell counts if the measurements are >20% different.

Scale Bio™ Quantum Barcoding Plate: Cell Loading

22. Dilute cells to 200 cells/µL using Hyb Buffer A and pipette mix to achieve a uniform cell suspension. Make a minimum of 1.7 mL to ensure 100 µL is available per Quantum Barcoding Plate well.
23. Using a multichannel pipette, completely remove Hyb Buffer A from each of the 16 wells of the Quantum Barcoding Plate by pipetting from a selected corner as shown in Figure 7.
24. Add 100 µL of cells (20,000 cells) ***dropwise*** with a 200 µL tip to the center of each of the 16 wells without touching the pipette to the bottom of the plate (see Figure 8 below). Gently vortex or pipette mix the cell suspension frequently to ensure uniform cell distribution across the wells.

Figure 8. When loading cells and beads into the Quantum Barcoding Plate, ensure that you are pipetting dropwise over the center of each well to allow for even distribution across the entire well.

25. Place the plate lid on the Quantum Barcoding Plate and let the cells settle for 5 min on a flat surface at RT.
26. Centrifuge the plate at ***600 x g for 3 min*** at RT with an appropriately weighted balance.
27. Gently remove the supernatant from the wells by pipetting from a selected corner of the well as shown in Figure 4.
28. Proceed immediately to Step 4: Bead Loading.

Step 4: Bead Loading, Hybridization and Collection

- Quantum Barcoding Bead Loading
- Hybridization
- Bead Collection

Step 4: Bead Loading, Hybridization and Collection

Before you begin:

- ***Set a swing bucket centrifuge to room temperature with adaptors that can hold 96-well plates.***
- ***Set an incubator to 37°C that will fit the Quantum Barcoding Plate.***

Please review the table below to prepare reagents before starting this protocol section:

Source	Consumable	Storage Temp	Place
TotalSeq™-A PhenoCyte - Module C	Quantum Barcoding Protein Beads	2°C to 8°C	Ice
	Hyb Buffer B	2°C to 8°C	Ice
	Bead Collection Buffer	2°C to 8°C	Ice
	Purification Buffer	2°C to 8°C	Ice
	Sample Collection Funnel	2°C to 8°C or RT	-
Other vendors	15-mL tube	-	-
Other vendors	2.0-mL DNA LoBind tube	-	-

Scale Bio™ Quantum Barcoding Protein Bead Loading

Procedure:

1. Gently pipette mix or vortex the Quantum Barcoding Protein Beads until the bead suspension is homogeneous before proceeding.
2. Aliquot 155 µL of Quantum Barcoding Protein Beads to a 2-mL tube and place on a magnetic stand.
3. Add 1 mL of Purification Buffer to the tube containing Quantum Barcoding Protein Beads on the magnetic stand.
4. Wait until the solution is clear.
5. With the 2-mL tube on the magnetic stand, remove all the supernatant using a pipette.
6. Repeat steps #3-5 twice more for a total of 3 washes.
7. Remove the 2-mL tube from the magnetic stand and resuspend the Quantum Barcoding Protein Beads in 1 mL of Purification Buffer.
8. Transfer the 1-mL of Quantum Barcoding Protein Beads into a 15-mL tube.
9. Add 4,120 µL of Purification Buffer to the 15-mL tube for a final volume of 5,210 µL.
10. Gently vortex the Quantum Barcoding Protein Beads until the bead suspension is homogeneous.
11. Load 150 µL Quantum Barcoding Protein Beads dropwise with a 200 µL tip to the center of each of the 16 wells of the plate without touching the pipette to the bottom of the plate as shown in Figure 8. Frequently vortex the bead suspension remaining in the 15-mL tube to ensure uniform bead distribution across the 16 wells of the Quantum Barcoding plate.

Note: It is recommended to aliquot the Quantum Barcoding Protein Beads into a reservoir for loading the plate with a multichannel pipette, making sure to keep the beads in a homogeneous suspension while loading.

12. Place the plate lid on the Quantum Barcoding Plate and let the Quantum Barcoding Protein Beads settle down in the microwells for 5 min on a flat surface at RT.
13. Centrifuge the plate at ***300 x g for 2 min*** at RT to load the Quantum Barcoding Protein Beads into the microwells with an appropriate balance.
14. Gently remove the supernatant from the wells by pipetting from a selected corner of the wells as shown in Figure 4.
15. Repeat steps #11-14 once more for a total of two bead loadings.
16. Gently add 150 µL of Purification Buffer to each of the wells by dispensing the liquid on the side walls of the wells.

    Note: It is recommended to aliquot Purification Buffer into a reagent reservoir for loading the plate with a multichannel pipette.

17. Slowly remove all the supernatant by pipetting from a selected corner of the well. Excess beads will be removed.
18. Repeat steps #17-18 twice for a total of three washes. Proceed immediately with Bead Hybridization.

Hybridization

19. Load 150 µL Hyb Buffer B buffer to each of the 16-wells by dispensing the liquid on the side walls of the wells.

    Note: It is recommended to aliquot Hyb Buffer B into a reagent reservoir for loading the plate with a multichannel pipette.

20. Seal the wells with a microfilm seal.
21. Place the plate lid on the Quantum Barcoding Plate and incubate the plate at ***37°C for 30 min***. .
22. Slowly remove *all* the supernatant from the wells by pipetting from a selected corner of the well as shown in Figure 4. Proceed immediately with Bead Collection.

Bead Collection

23. Add 100 µL of Bead Collection Buffer to each of the 16 wells by dispensing the liquid on the side walls of the wells.

    Note: It is recommended to aliquot Bead Collection Buffer into a reagent reservoir for loading the plate with a multichannel pipette.

24. Centrifuge at ***300 x g for 2 min at RT*** with an appropriate balance.
25. Remove the Sample Collection Funnel from its packaging. Do not touch the inside of the Sample Collection Funnel.
26. Place the Sample Collection Funnel upside down on top of the Quantum Barcoding Plate and quickly invert the assembly, as shown in Figure 9 below.

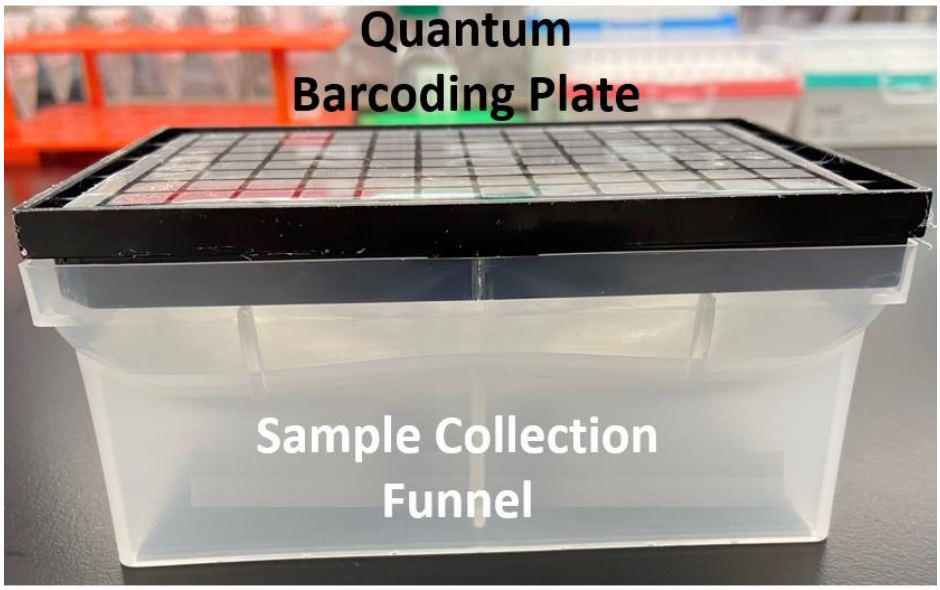


Figure 9. Placing the Sample Collection Funnel on top of the Quantum Barcoding Plate and inverting for bead collection .

27. Centrifuge the Sample Collection Funnel and Quantum Barcoding Plate with an appropriately weighted balance at ***300 x g for 3 min at RT.***
28. Carefully remove the upside-down Quantum Barcoding Plate from the Sample Collection Funnel and place the Quantum Barcoding Plate right-side up on a benchtop.
29. Collect the pooled Quantum Barcoding Protein Beads from the bottom of the Sample Collection Funnel and add to a 15-mL tube.
30. Place the 15-mL tube containing the Quantum Barcoding Protein Beads on a magnetic stand.
31. Wash the now empty Sample Collection Funnel with 2 mL of Purification Buffer, rinsing the sides of the Sample Collection Funnel. Ensure all liquid is removed.
32. Transfer the wash to the 15-mL tube containing Quantum Barcoding Protein Beads on the magnet.
33. Add 150 µL of Purification Buffer to each of the 16 wells by dispensing the liquid on the side walls of the wells.
34. Place the empty Sample Collection Funnel upside down on top of the Quantum Barcoding Plate and quickly invert the assembly.
35. Centrifuge the Sample Collection Funnel and Quantum Barcoding Plate with an appropriately weighted balance at ***300 x g for 3 min at RT.***
36. Collect the pooled Quantum Barcoding Protein Beads from the bottom of the Sample Collection Funnel and add it to the 15-mL tube containing the Quantum Barcoding Protein Beads on the magnetic stand.
37. Wash the now empty Sample Collection Funnel with 2 mL of Purification Buffer, rinsing the sides of the funnel.
38. Repeat steps #33-37 for a total of two washes.
39. Wait until the solution containing the Quantum Barcoding Protein Beads in the 15-mL tube on the magnetic stand becomes clear.
40. Discard the supernatant.
41. Add 2 mL of Purification Buffer to the 15-mL tube containing the Quantum Barcoding Protein Beads.

42. Gently vortex the tube and then put it back on the magnetic stand.
43. Discard the supernatant.
44. Repeat steps #41-43 for a total of two washes.
45. Remove the tube from the magnetic stand and resuspend the beads in 1 mL of Purification Buffer.
46. Transfer all the beads into a new 2.0-mL DNA LoBind tube.
47. Add 1 mL of Purification Buffer to the 15-mL tube and vortex lightly to collect any leftover Quantum Barcoding Protein Beads.
48. Transfer the leftover beads to the 2.0-mL DNA LoBind tube containing beads.

Step 5: Gap-Fill Ligation and Capture Bead Treatment

- Gap-Fill Ligation (GFL) Master Mix Preparation
- Gap-Fill Ligation Reaction
- Bead Treatment

Step 5: Gap-Fill Ligation and Capture Bead Treatment

Before you begin:

- ***Thaw the GFL Additive, GFL Buffer A and GFL Buffer B at Room Temperature.***

Please review the table below to prepare reagents before starting this protocol section:

Source	Consumable	Storage Temp	Place
TotalSeq™-A PhenoCyte - Module B	GFL Additive	-30°C to -10°C	RT
	GFL Buffer A	-30°C to -10°C	RT
	GFL Buffer B	-30°C to -10°C	RT
	GFL Enzyme A	-30°C to -10°C	Ice
	GFL Enzyme B	-30°C to -10°C	Ice
TotalSeq™-A PhenoCyte - Module C	Treatment Solution	2°C to 8°C	Ice
	Purification Buffer	2°C to 8°C	Ice
Other Vendors	Nuclease-Free Water	-	-
Other Vendors	1.5-mL tube	-	-

GFL Master Mix Preparation

Procedure:

1. On ice, prepare the GFL Master Mix by adding the components in the order presented in the following table to a 1.5-mL tube.

Component	1 Reaction + 10% (µL)
Nuclease Free Water	448.8
GFL Additive	8.8
GFL Buffer A	26.4
GFL Buffer B	26.4
GFL Enzyme A	8.8
GFL Enzyme B	8.8
Total	528

2. Gently pipette mix the GFL Master Mix. Briefly spin down and place the tube containing the master mix on ice.

Gap Fill Ligation Reaction

3. Place the 2.0-mL DNA LoBind tube containing Quantum Barcoding Protein Beads onto a magnetic stand.
4. When the solution becomes clear, remove all of the supernatant and then remove the 2.0-mL tube from the magnetic stand.
5. Add 480 µL of the GFL Master Mix to the 2.0-mL tube containing beads and resuspend the beads by gently pipette mixing until the bead suspension is homogeneous.
6. Incubate at room temperature for 30 minutes on a hula/rotator/orbital mixer.

! *Set the hula/rotator/orbital mixer speed and rotation so beads do not settle.*

7. After incubation, briefly spin down the tube.

Bead Treatment

Procedure:

8. In a new 1.5-mL tube, combine 85 µL of Treatment Solution with 765 mL of nuclease free water.
9. Briefly vortex the Treatment Solution and spin down.
10. Place the 2.0-mL DNA LoBind tube containing beads on a magnetic stand.
11. Wait until the solution becomes clear and then remove the supernatant.
12. Add 800 µL of prepared Treatment Solution to the 2.0-mL tube to resuspend the beads.
13. Gently pipette mix and then briefly spin down.
14. Incubate at RT for 5 min off the magnetic stand.
15. Place the 2.0-mL tube on a magnetic stand.
16. Wait until the solution becomes clear and then remove the supernatant.
17. Leaving the tube on the magnet, add 1 mL of Purification Buffer to the tube.
18. Wait 30 seconds for the Quantum Barcoding Protein Beads to separate.
19. With the tube on the magnet, remove the supernatant.
20. Taking the tube off the magnet, resuspend the Quantum Barcoding Protein Beads with 1 mL Purification Buffer.

Safe stopping point: Following Bead Treatment, Quantum Barcoding Protein Beads can be stored at 4°C for up to overnight in Purification Buffer.

Step 6: Library Amplification

- PCR Master Mix Preparation
- Library Amplification

Step 6: Library Amplification

Programming the PCR machine

Set the following program on PCR machine with a 105°C lid and a 50 µL volume.

Temp	*Time*	*Cycles*
98°C	∞	
98°C	45 sec	
98°C	15 sec	15 cycles
60°C	30 sec	
72°C	30 sec	
72°C	1 min	
10°C	∞	

Please review the table below to prepare reagents before starting this protocol section:

Source	Consumable	Storage Temp	Place
TotalSeq™-A PhenoCyte - Module B	PCR Master Mix	-30°C to -10°C	Ice
	Library Oligos	-30°C to -10°C	Ice
TotalSeq™-A PhenoCyte - Module C	Purification Buffer	2°C to 8°C	Ice
Other Vendors	Nuclease-Free Water	-	-
Other Vendors	0.2-mL PCR 8-strips Or 96-well plate	-	-

PCR Master Mix Preparation

Procedure:

1. On ice, prepare the PCR Master Mix by adding the components in the following table to a 1.5-mL tube.

Component	Volume (µL) per well	16 Reactions + 10% (µL)
PCR Master Mix	25	440
Nuclease-Free Water	22	387.2
Library Oligos	3	-
Total	50	827.2

2. Gently pipette mix the PCR Master Mix until the solution is homogeneous. Briefly spin down and place the tube on ice.

Library (PCR) Amplification

3. Pipette mix the Quantum Barcoding Protein Beads from the previous section to achieve uniform bead suspension.
4. Pipette 62.5 µL of Quantum Barcoding Protein Beads into two columns (16 wells) of a 96-well PCR plate or two 8-tube strips. Ensure all beads are transferred to the plate and pipette mix the stock frequently to ensure a uniform distribution of beads.
    a. Wash the now empty 2.0-mL tube with 1.0-mL of Purification Buffer and collect any remaining beads. Distribute 62.5 uL across the 16 wells/tubes.
5. Place the plate with Quantum Barcoding Protein Beads onto a magnet and wait until the solution is clear.
6. Remove the supernatant while the tubes are on the magnet.
7. Add the prepared master mix into a reservoir. Remove the plate from the magnet and add 47 µL of the prepared master mix to each well.
8. Add 3 µL of a unique Library Oligo into each well of the plate containing Quantum Barcoding Protein Beads.
9. Gently pipette mix to ensure beads are in suspension and proceed immediately to the next step.
10. Place the PCR plate in the pre-warmed thermocycler.
11. Run the thermocycler following the program below, ensuring to skip the first holding step.

Temp	***Time***	***Cycles***
98°C	∞	
98°C	45 sec	
98°C	15 sec	15 cycles
60°C	30 sec	
72°C	30 sec	
72°C	1 min	
10°C	∞	

Safe stopping point: Following PCR amplification, libraries can be stored at 4°C for up to 72 hours.

Step 7: Library Purification and QC

- Library Purification
- Post Amplification Library QC

Step 7: Library Purification and QC

Before you begin:

- ***Prepare 4-mL of fresh 80% ethanol.***

Please review the table below to prepare reagents before starting this protocol section:

Source	Consumable	Storage Temp	Place
Beckman Coulter	SPRI Beads	2°C to 8°C	RT
Other vendors	Freshly made 80% Ethanol	-	-
Other vendors	Elution Buffer or 10mM Tris, pH 8.0	-	-
Other vendors	Magnetic stand (1.5-mL compatible)	-	-
Other vendors	1.5-mL Tubes	-	-
Other vendors	2.0-mL Tubes	-	-
Invitrogen	Qubit dsDNA HS Assay Kit	2°C to 8°C	RT
Agilent	D5000 ScreenTape Kit or equivalent	2°C to 8°C	RT

Library Purification

Procedure:

1. Pool 20 µL from each of the 16 wells of the PCR Amplification reaction into a 1.5-mL tube.
2. Vortex the SPRI Beads well until they appear homogeneous in color.
3. Add 320 µL of SPRI Beads (1.0X) to the tube containing PCR amplification products and pipette mix until the solution is homogeneous.
4. Incubate at room temperature for 5 min.
5. Briefly spin and place the tube on a magnetic stand for 2 min.
6. Keep the tube on the magnetic stand and remove the supernatant, being careful not to disturb the beads.
7. Add 1 mL of 80% ethanol to the side of the tube opposite of the pellet.
8. Incubate for 30 secs.
9. Discard the supernatant, being careful not to disturb the beads.
10. Repeat the 1 mL of 80% ethanol wash for a total of two washes.
11. Briefly spin the tube to collect the residual ethanol at the bottom of the tube and place it back on the magnetic stand.
12. Remove residual 80% ethanol, being careful not to disturb the beads.
13. Air dry the beads for 1 min or until the bead pellet appears matte instead of glossy but not cracked.
14. Remove the tube from the magnetic stand and add 50 µL of Elution Buffer.
15. Pipette mix to resuspend the beads in solution.
16. Incubate at room temperature for 2 mins.
17. Place the tube back on the magnet and wait until the solution is clear.

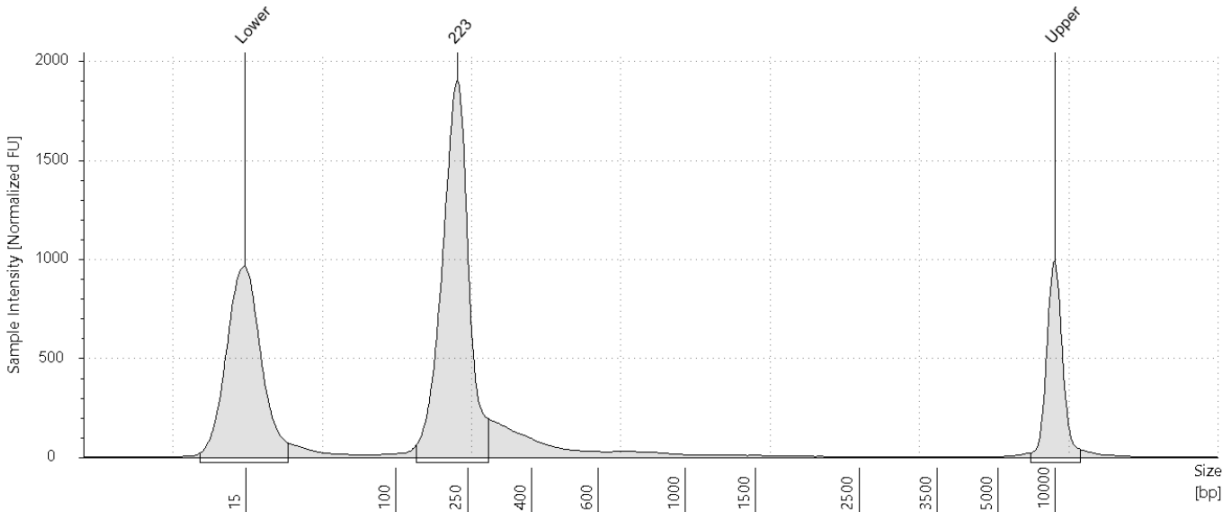
18. Transfer the supernatant into a fresh 1.5-mL tube.

Safe stopping point: Purified Libraries can be stored at -20°C for up to two months.

Post Amplification Library QC

1. Measure the library concentration with Qubit™ 1X dsDNA High Sensitivity.
    a. The library concentration with cryopreserved PBMCs is between 1-5 ng/µL. The concentration may vary depending on sample input.
2. Determine size of the library using an Agilent TapeStation or equivalent fragment analyzer.
    a. Amplicon size will be approximately 220 base pairs.

Example trace:



Library Sequencing and Data Processing

- Library Sequencing Requirements
- Data Processing and Analysis

Library Sequencing Requirements

Sequence the library on an Illumina sequencer using the following parameters. Perform library dilution and clustering according to sequencing manufacturers' parameters.

Parameter	Description
Sequencing Depth	10k Reads per cell
Sequencing Type	Dual indexed, pair-end
Supported Sequencers	Illumina® NovaSeq 6000 Illumina® NovaSeq X
Read Cycles	Number of Cycles Read 1: 45 Index 1: 16 Index 2: 8 Read 2: 15
PhiX Concentration *(per respective Illumina Denature and Dilute guide for the instrument)*	10% PhiX

Data Processing and Analysis

BCL to FASTQ Processing

The libraries are sequenced on Illumina instruments, which generate raw base call files (BCL) as the primary output. The per-cycle BCL files need to be translated to a per-read FASTQ file before proceeding with the Multiomics Analysis Software (MAS) pipeline. It is recommended to use Illumina's BCLConvert application for FASTQ conversion.

Note: Please refer to the Multiomics Analysis Software User Manual for detailed instructions on creating the sample sheet for the BCLConvert application or please reach out to BioLegend Technical Service for assistance.

Multiomics Analysis Software (MAS): FASTQ to Count Matrices and Data Visualization

BioLegend's cloud-Multiomics Analysis Software (MAS) can be used to perform FASTQ to count processing as well as downstream analysis including filtering, dimensionality reduction and implementing flow-like gating strategies. For detailed instructions on uploading and data visualization with the TotalSeq™ PhenoCyte kit, please refer to the MAS user guide.

Appendix

- Oligonucleotide Indexing Sequences
- Quantum Barcoding and Indexing Sequences
- FAQs
- Troubleshooting

Appendix

Oligonucleotide Indexing Sequences

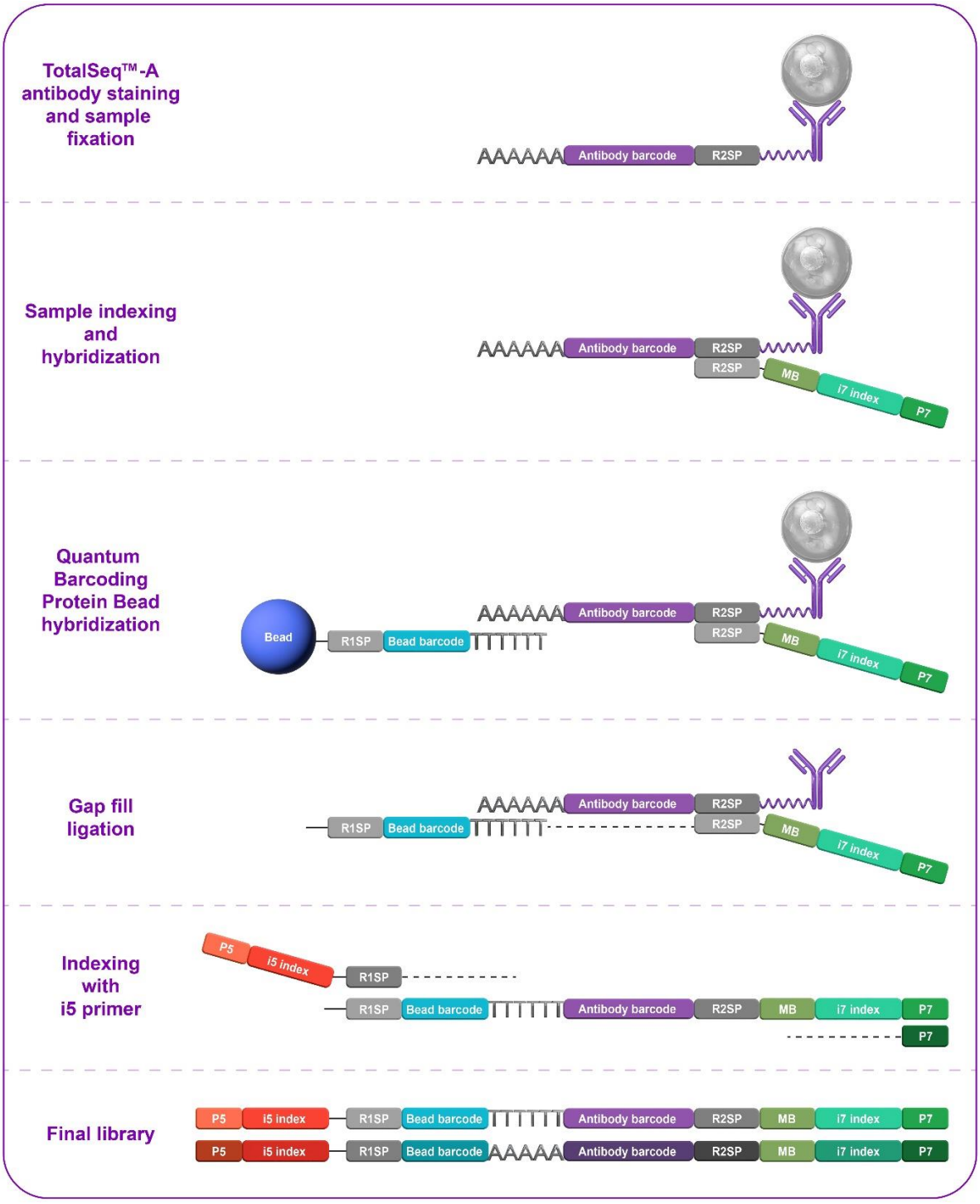
Sample Indexing Oligo Sequences (I7 Indices)
PN 750004351

Well	i7 Index Sequence	Sample Identifier
A1	ATATCACG	
B1	ACATTAGT	
C1	TGATGTGC	
D1	AGAGTTGG	
E1	AGCTTCGT	
F1	AATGTATG	
G1	GAACAGCA	
H1	ACCTTCTC	
A2	TCCTCGAT	
B2	ATGACAAG	
C2	AACAGACA	
D2	GTCGTATC	
E2	AGTAAGCC	
F2	CTTGAGCA	
G2	TGTATGTC	
H2	CCAATAGG	

Library Oligo Sequences (I5 Indices)
PN 750004348

Well	I5 Index Forward equence	I5 Reverse Compliment Sequence
A1	TTGGATCG	CGATCCAA
B1	TGAATGGC	GCCATTCA
C1	ACCTGTAC	GTACAGGT
D1	CTCGACAA	TTGTCGAG
E1	GTTGACAC	GTGTCAAC
F1	ACAAGGAC	GTCCTTGT
G1	ACACCTTG	CAAGGTGT
H1	TGTGGTAG	CTACCACA
A2	CATACCGT	ACGGTATG
B2	TCAGGCAT	ATGCCTGA
C2	CAGAGGAA	TTCCTCTG
D2	GATGTCTC	GAGACATC
E2	TTGAGACC	GGTCTCAA
F2	TCTTCACC	GGTGAAGA
G2	TGTGAGCT	AGCTCACA
H2	CAACGATC	GATCGTTG

Scale Bio™ Quantum Barcoding and Indexing Sequences



The cell barcode used to identify individual cells is made up of a unique combination of the i7 sample index and the bead barcode. For the 200k kit size there are 16 i7 indices and 880,000 unique bead barcodes or ~14x10^6 possible sample index/bead barcode combinations.

FAQs

Can I use part of the plate and save the other wells for a future experiment?

The kit design with cell barcoding requires all components be used in one experiment.

What do I do if I am hashing my cells?

Combine the cells after resuspension in Hyb Buffer A and then quantify the cells before sample indexing per the protocol. Perform the protocol as instructed, cell hashing barcodes will be detected in the final sequencing output.

What is the expected cell recovery?

The expected cell recovery is greater than 62.5% when loading at the recommended cell density.

Should I use hashing or the sample indexes for sample identification?

We recommend using TotalSeq Hashtags to multiplex samples since sample indexing may require the use/purchase of additional TotalSeq cocktails.

Can I use both cell hashing and sample indexing to multiplex my samples?

Although possible, we do not recommend combining both hash and sample (i7) multiplexing within the same experiment. Please reach out to BioLegend Technical support before proceeding and for additional guidance. Note that guidance for this approach is limited.

At what point can I index my samples without cell hashing?

Samples can be indexed with the sample indexing oligos. The 200k throughput kit comes with 16 unique indexes to choose from. The 1.2M throughput kit comes with 24 unique indexes to choose from. This may require the use/purchase of additional TotalSeq cocktails depending on the number of samples and kit size used.

What if I do not have 20,000 fixed, stained and indexed cells to load into each well of the Quantum Barcoding Plate?

Fewer cells may be loaded into the wells, but we have only validated down to 16,000 cells. Fewer than 16,000 cells have not been tested. The number of overall cells recovered will be lower in these cases.

Can I load more than 20,000 fixed, stained and indexed cells into the Quantum Barcoding Plate?

Loading more cells will increase the multiplet rate of the data. More than 20,000 cells has not been tested.

What indexes do I need to keep track of?

The sample index (i7) is the only index that is required to be tracked if processing multiple samples. Even with multiple samples, the PCR oligos (i5) do not need to be tracked as that is a part of the cell barcode.

My library yield is different than the expected amount.

Expected library yields are an average for cryopreserved PBMCs and will differ depending on cell types. Different cell inputs will also result in different yields.

Troubleshooting

I do not have a final library. What happened?

Multiple failure points can result in a failure to produce a library.

- Ensure the staining and fixation process was completed properly.
- Ensure Quantum Barcoding Plate was both wetted and washed.
- Ensure the Sample Indexing Oligos were not mixed up with the Library Oligos.
- Ensure all GFL reagents were added to the master mix.
- Ensure treatment solution was properly diluted and used to treat the beads. Failure to treat the beads can result in no final library.
- Verify the PCR thermocycling program was completed to the specified program.

This is not an exhaustive list of all possible failure modes but the most common. If the protocol was followed correctly, re-pool the library from the final PCR and perform the purification again to ensure the library was not lost during the bead clean up.

Low cell recovery

Verify that the cell count before loading into the Quantum Barcoding Plate wells was within 20% of the duplicate counts. Incorrect cell loading will result in variable cell recovery.

Low cell recovery post sample indexing

Expected cell recovery is greater than 62.5% from the cells inputted into sample indexing. Verify spin bucket centrifuges were used and tubes containing stained and fixed cells were homogenous before pooling together into one tube. Also ensure the aspiration of supernatant was angled away from the expected cell pellet location.

High multiplet rate

Verify that the cell count before loading into the Quantum Barcoding Plate wells was within 20% of the duplicate counts. Loading more than 20,000 cells per well will result in a higher multiplet rate.

Low valid barcode percentage (<80%)

Ensure 10% PhiX is loaded with sequencing to improve diversity on the flowcell.

For any issues, questions, or feedback please reach out to BioLegend Technical Services:

Phone Toll-Free (US & Canada): 1.877.273.3103

Phone (International): 1.858.768.5801

For email inquiries, please use the following form: https://www.biolegend.com/en-us/contact-technical-service