
tapestri

# Antibody Hashing for Tapestri® Single-Cell DNA Sequencing

## User Guide

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# Introduction

The *Mission Bio Tapestri® Platform* uses microfluidic droplet technology to combine single-cell lysates with barcoding beads and gene specific primers to deliver a high-throughput single-cell genomics workflow for targeted DNA sequencing. Users can produce a sequencing-ready library starting from a single-cell suspension in as few as 2 days. The cell preparation procedures described in this User Guide allow for sample multiplexing by staining each cell suspension with unique hashtag antibody-oligo conjugates (AOCs) and then combining them in a single Tapestri run. With this approach, users can process multiple samples simultaneously, including from related individuals or from cell lines with a common genetic background. Cells belonging to each sample can be demultiplexed using the Tapestri Pipeline. For accurate demultiplexing, each sample must comprise at least 0.1% of the pooled cell suspension.

# About This Guide

Here we introduce a detailed experimental procedure for preparation of single-cell suspensions from up to three independent samples, staining with hashtag AOCs, optional co-staining with any lyophilized TotalSeq™-D AOC cocktail, and subsequent sample multiplexing prior to single-cell library preparation on the Mission Bio Tapestri® instrument. This protocol has been validated for human cell lines, peripheral blood mononuclear cells (PBMCs), and bone marrow mononuclear cells (BMMCs). Following thawing, staining, and sample pooling, users should refer to the *Tapestri® Single-Cell DNA + Protein v3 User Guide (PN MB05-0018)* for subsequent protocol steps, starting with *Chapter 2: Encapsulate Cells.*

# Materials

## Tapestri® Single-Cell DNA + Protein Kit Components

| Component Name | Kit | Storage |
|---|---|---|
| ○ Blocking Buffer (white cap) | Tapestri® Protein Staining Kit v3 | 4 °C |
| Cell Buffer | Tapestri® Single-Cell DNA Core +4 Kit v3 | 4 °C |

## Required Third Party Consumables and Reagents

| Component Name | Supplier (Part Number) | Protocol Step |
|---|---|---|
| TipOne RPT ultra low retention filter tip | USA Scientific (1180-8810) or Approved Supplier | Reagent handling |
| 1.5 mL Protein LoBind tubes | Eppendorf (22431081) or Approved Supplier | Reagent handling |
| 200 µL Wide bore tip, rack, sterile<br>1000 µL Wide bore tip, rack, sterile | USA Scientific (1011-8410),<br>USA Scientific (1011-9410) or Approved Supplier | Cell handling |
| 15 mL DNA LoBind conical tubes | Eppendorf (30122208) or Approved Supplier | Cell handling |
| 1.5 mL DNA LoBind Microcentrifuge Tubes | Eppendorf (0030108035) or Approved Supplier | Cell handling |
| Flowmi™ Cell Strainers for 1000 µL pipette tips, 40 µm | Fisher Scientific (14-100-150) | Cell handling |
| Sterile single-pack CellTrics™ filters, 30 µm (OPTIONAL) | Sysmex (04-004-2326) | Cell thawing |
| Fetal Bovine Serum (FBS) | ATCC (30-2021) or Approved Supplier | Cell thawing |
| Propidium Iodide (PI) | Thermo Fisher (P3566) or Approved Supplier | Cell quantification |
| Trypan Blue (OPTIONAL) | Thermo Fisher (15250061) or Approved Supplier | Cell quantification |
| TotalSeq™-D0251 anti-human Hashtag 1 | BioLegend (394602) | Cell staining |
| TotalSeq™-D0252 anti-human Hashtag 2 | BioLegend (394604) | Cell staining |
| TotalSeq™-D0253 anti-human Hashtag 3 | BioLegend (394606) | Cell staining |
| Human TruStain FcX™ (Fc Receptor Blocking Solution) | BioLegend (422301) | Cell staining |
| Cell Staining Buffer (CSB) | BioLegend (420201) | Cell staining |
| TotalSeq™-D AOC Cocktail (OPTIONAL) | BioLegend (399906),<br>Mission Bio (MB03-0102, -0105, -0121) | Cell staining |

**NOTE** ***Unless otherwise noted, do not substitute. Only listed consumables have been validated by Mission Bio.***

## Required Benchtop Equipment

| Equipment | Supplier (Part Number) |
|---|---|
| Pipettes, 1 µL – 1000 µL | Mettler-Toledo, Rainin Pipettes, or Approved Supplier |
| Microcentrifuge (1.5 mL PCR tubes) with temperature control | Thermo Fisher (5406000240) or Approved Supplier |
| Tube Vortexer | Thermo Fisher (88880017TS) or Approved Supplier |
| Centrifuge with temperature control and swinging bucket (needs to support 15 mL and 1.5 mL tubes) | Eppendorf (5810 R) or Approved Supplier |
| Countess™ II Automated Cell Counter or equivalent | Thermo Fisher (AMQAX1000) |

# Cell Handling Guidelines

The steps provided in this protocol are applicable to hashtag labeling of human cell lines, PBMCs and BMMCs. Different sample types may require revised procedures including cell thawing, washing, labeling, or quantification.

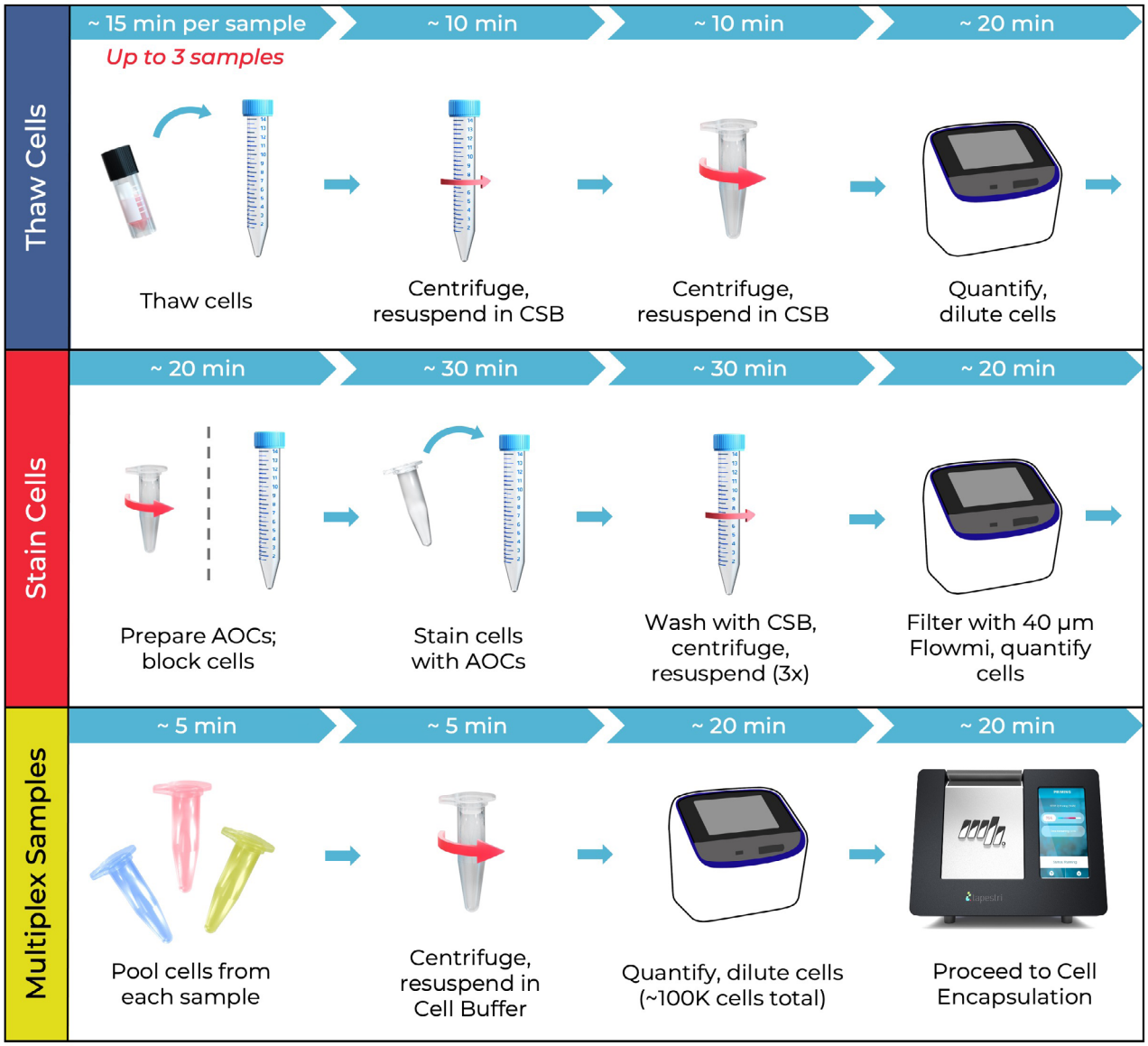
## Cell handling

- Avoid the use of samples containing significant debris, dead cells, or fragments of lysed cells.
- For optimal performance, it is important to obtain a single-cell suspension before cell staining. Pass cells through a 30 µm filter to remove cell clumps during thawing and manually remove cell clumps throughout the process if observed.
- The workflow is optimized for a starting cell number of 0.5–1 million. Using a cell input number outside of this range may result in insufficient cell recovery or cell staining.
- Using a fixed rotor centrifuge may result in cell loss due to insufficient cell pelleting. It is strongly recommended to use a centrifuge with swinging buckets that supports 15 mL and 1.5 mL tubes to maximize cell recovery.
- Use of wide bore tips to mix and resuspend cells is recommended to maintain cell viability.
- When not actively handling cells, store on ice.

## Cell quantification and viability

- Mission Bio strongly recommends the use of an automated cell counter, such as the Countess™ Automated Cell Counter (Thermo Fisher ).
- Mission Bio strongly recommends the use of fluorescent exclusion reagents such as Propidium Iodide (PI) to determine cell death/viability. PI-based assays compared to Trypan Blue-based assays are more robust in accurately determining the percentage of dead/viable cells. Please follow the manufacturer's instructions when using PI-based viability assays.
- Cell suspensions should have a viability > 80% prior to cell staining. If viability is lower, dead cell removal is recommended.
- Final cell concentration values are based on the **total (live + dead)** cell counts.
- Following cell staining, up to three samples may be multiplexed for one Tapestri run.

# Protocol Overview




*Total time until stopping point: ~ 4 hours*

# Cell Preparation Protocol

## 1. Prepare Cell Suspension

# Antibody Hashing Protocol

## 1. Prepare Cell Suspension

This section describes the steps required to prepare a single-cell suspension, stain cells with TotalSeq™-D AOCs, and pool stained cells from up to three samples. The workflow is optimized for a starting cell number of 0.5 x $10^6$ to 1 x $10^6$ at greater than 80% viability in Cell Staining Buffer (BioLegend) with a minimum volume of 40 µL. Some cell loss is to be expected throughout the staining and washing procedure and therefore a recommended 12,500 – 25,000 cells/µL ensures sufficient cell recovery for downstream sample multiplexing.

**NOTE**

- ***Thaw reagents at room temperature unless directed to thaw them on ice.***
- ***Store reagents according to manufacturer's storage recommendations as soon as they are received. Vortex and then centrifuge reagents as directed.***
- ***The following procedure assumes cell lines, PBMCs or BMMCs to be cryopreserved in 2 mL cryovials in a total volume of 0.5 mL and stored in liquid nitrogen or -80°C.***
- ***If starting with cells cultured in growth media, transfer cells to a 15 mL conical tube and proceed from Step 1.14.***

### Thaw Cells

1.1 Retrieve all reagents required for preparing the cell suspension:

- Cell Buffer (4 °C, +4 Kit) → place on ice
- Human TruStain FcX™ (BioLegend) → place on ice
- Blocking Buffer (●) (4 °C, Protein Staining Kit) → place on ice
- Cell Staining Buffer (CSB, BioLegend) → place on ice
- TotalSeq™-D Hashtags 0251-0253 (BioLegend) → place on ice
- Flowmi Cell Strainer
- (OPTIONAL) TotalSeq™-D AOC cocktail → keep at RT

1.2 **Warm thawing media** (for instance 40% FBS + 60% base media) to 37 °C.

**IMPORTANT** ***Perform Steps 1.3–1.9 for each sample one at a time. Samples can be processed side-by-side for the remaining steps.***

1.3 Remove cryovial of cells from liquid nitrogen or the -80 °C freezer, **immediately transfer** to a biosafety hood, twist the cap a quarter to relieve pressure, and immediately retighten.

1.4 **Immediately transfer to a 37° C water bath**, quickly thaw the vial by gently swirling the tube until a small amount of ice remains (< 1 minute). Be sure to avoid submerging the tube completely.

1.5 Remove tube and clean with 70% ethanol.

1.6 Using aseptic techniques, add **1 mL of pre-warmed thawing media drop-wise** to the cryovial. Transfer the entire contents of the vial to a 15 mL conical tube.

1.7 Using a wide bore P-1000 tip, **rinse** the vial with **1 mL of pre-warmed thawing media.** Transfer to the 15 mL conical tube containing the cells, drop by drop, making sure to pipette against the wall. Gently shake tube while adding.

1.8 Add **2 mL of thawing media** to 15 mL tube, drop by drop, making sure to pipette against the wall, and gently shake the tube while adding.

1.9 **Add 0.5 mL of thawing media** to 15 mL tube **every few seconds until 12 mL total volume** is reached. Gently mix the tube by hand after each addition.

**IMPORTANT** ***If processing multiple samples, place the 15 mL tube on ice and repeat Steps 1.3–1.9 for the next sample until all samples have been thawed.***

1.10 Centrifuge at **400 x g for 5 minutes** at room temperature.

1.11 Immediately aspirate supernatant, leaving 0.5 mL to 1 mL of thawing media behind. **Do not disturb the cell pellet.**

1.12 Using a wide bore tip, gently **resuspend the cell pellet** in remaining thawing media by pipetting up and down 5x.

1.13 Add 10 mL of thawing media to the tube.

1.14 Centrifuge at **400 x g for 5 minutes** at room temperature.

1.15 Immediately aspirate all supernatant. **Do not disturb the cell pellet**.

1.16 Using a wide bore P-1000 tip, **resuspend the cell pellet in 1 mL of CSB**.

1.17 ***OPTIONAL*** (If using a diploid cell line as a control for CNV measurement): **Quantify the resuspended cells** using an automated cell counter or hemocytometer following best practices and the manufacturer's instructions. **Quantify the diploid cells and add enough diploid cells to constitute 5% of the cell suspension.**

1.18 Centrifuge at **400 x g for 5 minutes** at room temperature.

1.19 Immediately aspirate all supernatant. **Do not disturb the cell pellet**.

1.20 Resuspend the cells in **50 µL of CSB**.

1.21 **Quantify the cells and assess viability** using an automated cell counter or hemocytometer following best practices and the manufacturer's instructions.

1.22 Dilute cell suspension to **12,500–25,000 cells/µL using CSB** in a **minimum volume of 40 µL**.

1.23 **Store the cells on ice** until used for staining the cells (*Step 1.28*) and proceed **immediately** to *Step 1.24*.

**IMPORTANT** ***Cells must not be stored longer than 30 minutes as a subset of cells (e.g., monocytes) are prone to stick to the tube plastic and may be unrecoverable.***

## Prepare Hashtag Antibody-Oligo Conjugates (AOCs)

The TotalSeq™-D hashtags 0251-0253 (BioLegend) are supplied at 0.5 µg/µL. The hashtags require dilution prior to staining the cells. Dilutions should be prepared fresh immediately preceding cell staining.

1.24 Briefly **vortex the hashtag AOC tubes** and quick-spin to collect the contents.

1.25 For each hashtag, combine **124 µL of CSB and 1 µL of hashtag in a new 1.5 mL Protein LoBind tube**. Briefly vortex and quick-spin to collect the contents.

**NOTE** ***Individual samples can exhibit variability in antibody affinity and specificity, leading to variations in staining efficiency and background. In some cases, the hashtag concentration values may need to be adjusted. Contact support@missionbio.com for more information.***

**IMPORTANT** ***Perform Steps 1.26 a – g in parallel for up to three vials of lyophilized AOC cocktail, depending on how many samples are being processed.***

1.26 ***OPTIONAL*** (If co-staining with any lyophilized TotalSeq™-D AOC cocktail):

- a. Retrieve a vial of the room temperature-equilibrated TotalSeq™-D AOC cocktail.
- b. Centrifuge the tube at **10,000 x g for 30 seconds** at room temperature.
- c. Add **45 µL of CSB** (BioLegend) **and 15 µL of the 1:125 hashtag dilution** from *Step 1.25* above to the lyophilized panel.
- d. Close the tube with the original cap and **vortex for 10 seconds**.
- e. Incubate at **room temperature for 5 minutes**.
- f. **Vortex** the tube for **10 seconds** and centrifuge at **10,000 x g for 30 seconds** at room temperature.
- g. **Transfer the entire volume (60 µL)** of reconstituted panel to a **Protein LoBind** Eppendorf tube.

1.27 Centrifuge the tubes (from either *Step 1.25* or *Step 1.26 g* above) at **14,000 x g for 15 minutes at 4° C**. Once completed, the AOCs must be used immediately in *Step 1.30*. In the meantime, proceed to the next step.

## Stain Cells

1.28 In a 15 mL DNA **LoBind** conical tube add the following reagents:

| Reagent | Volume (µL) |
|---|---|
| Cell Suspensions in CSB (12,500 –25,000 cells/µL) | 40.0 |
| Blocking Buffer ( ) | 5.0 |
| Human TruStain FcX™ | 5.0 |
| **Total Volume** | **50.0** |

**Table 1.** Reagents to block cells.

1.29 **Gently mix** by pipetting up and down with a wide bore P-200 tip and **incubate the solution for 15 minutes on ice**.

1.30 After 15 minute incubation, using a P-200 tip, **aspirate 50 µL** of the centrifuged AOCs and **add to the blocked cell suspension**. Cell staining solution total volume is 100 µL.

**IMPORTANT** ***Avoid touching the bottom or sides of the tube containing the AOCs with the pipette tip to avoid pelleted protein aggregates. Aggregates are not visible.***

1.31 **Gently mix** by pipetting up and down with a wide bore P-200 tip.

1.32 **Incubate cell staining solution for 30 minutes on ice.**

1.33 **Add 14 mL of pre-chilled CSB** to the cell staining solution, pipetting against the tube wall.

1.34 Centrifuge at **400 x g for 10 minutes at 4° C** in a swinging bucket.

1.35 Carefully **aspirate and discard 13.5 mL** of supernatant using a serological pipette.

**IMPORTANT** ***Aspirate from the top of the solution and avoid touching the bottom and sides of the tube. Leave at least 0.5 mL of supernatant behind. Do not disturb or resuspend the cell pellet. Cell pellet may not be visible.***

1.36 **Repeat** steps ***1.33*** to ***1.35*** for two additional washes, centrifuging at **400 x g for 5 minutes each at 4° C**.

1.37 **Remove and discard supernatant, leaving ~100 µL:** Aspirate all but 1 mL of supernatant using a serological pipette, then switch to a P-1000 tip to remove the remaining supernatant (~900 µL).

1.38 **Add 400 µL of CSB** to the cell pellet and resuspend by gently pipetting up and down several times using a wide bore P-1000 tip. Total volume is ~500 µL.

1.39 **Filter the cells with a 40 µm Flowmi cell strainer:** Aspirate 500 µL of the cell suspension with a P-1000 tip, place strainer on pipette tip, and dispense the liquid through the strainer into a new 1.5 mL DNA LoBind tube.

1.40 **Place on ice** and proceed to the next step.

## Quantify and Multiplex Samples

1.41 **Quantify the cells and assess viability** using an automated cell counter or hemocytometer following best practices and the manufacturer's instructions.

**NOTE** ***The recommended input for Cell Encapsulation on the Tapestri® instrument is 100,000 cells (30,000 minimum). It is recommended to use 150,000 cells in Step 1.42 below (50,000 minimum), to account for cell loss.***

1.42 **Pool cells** from up to three samples for a **total of 150,000 cells** in a new **1.5 mL DNA LoBind** tube. *If the volume of the multiplexed cell suspension is < 1 mL, add CSB to achieve a total volume of 1 mL.*

1.43 Centrifuge the multiplexed cell suspension at **400 x g for 5 minutes** at 4 °C in a swinging bucket.

1.44 Carefully remove all supernatant. **Do not disturb the cell pellet.**

1.45 Gently resuspend the cell pellet in **40 µL of Mission Bio Cell Buffer** by pipetting up and down several times with a wide bore P-200 tip.

1.46 **Quantify the cells and assess viability** using an automated cell counter or hemocytometer following best practices and the manufacturer's instructions.

1.47 If necessary, dilute cell suspension to **2,800–3,200 cells/µL** using Cell Buffer. **Confirm final concentration using a cell counter or hemocytometer.**

1.48 **Place cells on ice** and proceed **immediately** to Cell Encapsulation on the Tapestri® instrument (*Tapestri® Single-Cell DNA + Protein v3 User Guide, PN MB05-0018, Chapter 2: Encapsulate Cells*).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