# MultiPro® Human Discovery Panel Staining Protocol

## Cat No. G900150

### Required Reagents and Consumables

| Description | Manufacturer | Part Number |
|---|---|---|
| PBS, Ultrapure (1X) | Avantor | K812-500ML |
| PBS, Ultrapure (10X) | Invitrogen | AM9625 |
| Bovine Serum Albumin, Nuclease-free | Millipore Sigma | 126609100GM |
| Enhanced Blocking Reagent | Proteintech Genomics | G900005 |
| Protector RNase inhibitor | Roche | 3335399001 |
| Tween-20, 10% | Thermo Scientific | 28320 |
| NP-40, 10% | Thermo Scientific | 85124 |
| Dextran Sulfate Sodium Salt, 8 kDa | Millipore Sigma | RES2029D-A7 |
| Paraformaldehyde, 32% | Electron Microscopy Sciences | 15714 |
| Human TruStain FcX™ | BioLegend | 422302 |
| Protein LoBind Tube, 1.5 mL | Eppendorf | 022431081 |
| Nuclease-free Water | - | - |

### Required Equipment

- Centrifuge capable of 14,000 x g, with a rotor suitable for 1.5 mL microcentrifuge tubes.
- Swinging bucket centrifuge with adapter capable of holding 1.5 mL microcentrifuge tubes.
- Tabletop vortex mixer.

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### Workflow and Protocols

Please review the following protocols to ensure proper execution of each step in the entire workflow before starting.

**Cell hashing (Optional)**
C10014 – MultiPro HashMax™-5 Sample Multiplexing & Sequencing Library Prep Protocol for Flex

**Cell staining with the Human Discovery Panel**
This Document – C10013 (Rev B)Cell Staining (this protocol), followed by either:

Next GEM Flex**:** 10x Genomics Demonstrated Protocol for Cell Surface and Intracellular Protein Labeling for Fixed RNA CG000529 Rev C

GEM-X Flex: 10x Genomics Demonstrated Protocol for Cell Surface and Intracellular Protein Labeling for GEM-X Flex Gene Expression CG000781 Rev A

**Critical:** If using GEM-X Flex, follow recommended changes to this protocol as described in step 13 of this protocol (C10013, MultiPro Human Discovery Panel Protocol) to include an intracellular blocking step using 50 µL IC Block and Staining Buffer.

**10x Genomics Flex Fixation**

Next GEM Flex**:** 10x Genomics Fixation of Cells & Nuclei for Chromium Fixed RNA Profiling protocol (CG000478 Rev D).

GEM-X Flex: 10x Genomics Fixation of Cells & Nuclei for GEM-X Flex Gene Expression (CG000782 Rev B)

**10x Genomics Flex**

Next GEM Flex**:** Chromium Fixed RNA Profiling Reagent Kits for Multiplexed Samples with Feature Barcode technology for Protein using Barcode Oligo Capture

GEM-X Flex**:** GEM-X Flex Gene Expression Reagent Kit for Multiplex samples with Feature Barcode technology for Protein Expression CG000789 Rev A

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### Before You Start

**Time Required** - This protocol is expected to take ~3 to 4 hours after cell preparation, depending on the number of samples being stained. The staining protocol does not contain any safe stopping points and users must immediately proceed to sample fixation in the appropriate protocol listed above.

**Sample Preparation** - A high-quality sample is critical for assay performance. The ideal cell suspension has high viability (> 80%) and minimal cell clumps and debris. We strongly recommend straining cells through a 30 µm filter such as Pre-Separation Filters (Miltenyi PN: 130-041-407) to remove cell aggregates. During resuspension and mixing steps, pipette gently and avoid introducing bubbles.

**RNase Awareness** - To prevent RNA degradation, use nuclease-free reagents and consumables listed in this protocol. Before starting, decontaminate your work surfaces and pipettes using RNase Zap (Invitrogen PN: AM9780).

**Intracellular Staining** - For some sample types, it may be necessary to adjust the concentrations of detergent and fixative in the buffers listed below to achieve optimal results for intracellular staining.

**Centrifuge selection** - All steps requiring centrifugation of a cell suspension should be performed using a swinging bucket rotor centrifuge. Use of a fixed angle rotor can result in smearing of the cell pellet, which may negatively impact cell recovery.

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**Sequencing Parameters**

| For Cell Ranger v8.0 or newer | For Cell Ranger v7.2 |
|---|---|
| Read 1: 28 cycles | Read 1: 48 cycles |
| Index 1: 10 cycles | Index 1: 10 cycles |
| Index 2: 10 cycles | Index 2: 10 cycles |
| Read 2: 90 cycles | Read **2**: 50 cycles |

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## Buffer Preparation

### Prepare a 30% BSA Stock Solution

Dissolve 30 g of nuclease-free BSA powder in 100 mL of nuclease-free water. Filter-sterilize using a 0.2 µm filter and store at 4 °C for up to one week. When pipetting chilled 30% BSA, *slowly* aspirate and expel with your pipette to ensure proper delivery of required volumes.

### Prepare a 1% Dextran Sulfate Stock Solution

Dissolve 1 g of dextran sulfate, 8 kDa in 100 mL of nuclease-free water. Store at room temperature for up to 1 month.

### IC Block and Staining Buffer

**Critical:** If using GEM-X Flex, half of the prepared IC Block and Staining Buffer will be used to pre-block your cells in the 10x Genomics Demonstrated Protocol for Cell Surface and Intracellular Protein Labeling for GEM-X Flex Gene Expression CG000781 Rev A protocol. The other half will be used to reconstitute your antibodies. If you are staining using individual antibodies and not a lyophilized cocktail, use the "single sample + 10%" option when preparing your IC Block and Staining Buffer. Individual antibodies for intracellular staining will be diluted together in 55 µL of IC Block and Staining buffer.

IC Block and Staining Buffer is equivalent to "Buffer for Labeling (MultiPro Blocker)" in the 10x Genomics GEM-X Flex Protocol (CG000781 Rev A)

| Component | [Final] | 1 reaction + 10% (µL) | 4 reactions + 10% (µL) |
|---|---|---|---|
| RNase inhibitor (200X) | 1X | 1.1 | 4.4 |
| Nuclease-free BSA, 30% | 7.55% | 27.68 | 110.73 |
| Tween-20, 10% | 0.2% | 2.2 | 8.8 |
| NP-40 or Nonidet P40 Substitute, 10% | 0.5% | 5.5 | 22 |
| Dextran Sulfate, 8 kDa, 1% | 0.025% | 2.75 | 11 |
| Enhanced Blocking Reagent | - | 15.95 | 63.8 |
| Human TruStain FcX™ | - | 5.5 | 22 |
| PBS, Ultrapure (10X) | 1x | 11 | 44 |
| Nuclease-free Water | - | 38.32 | 153.27 |
| Total | | **110 µL** | **440 µL** |

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**BSA Buffer**

| Component | [Final] | Per sample (mL) | 4X samples (mL) | 8X samples (mL) |
|---|---|---|---|---|
| BSA, 30% | 1% | 0.11 | 0.44 | 0.88 |
| PBS, Ultrapure (10X) | 1X | 0.330 | 1.32 | 2.64 |
| Nuclease-free water | | 2.86 | 11.44 | 22.88 |
| Total | | 3.3 mL | 13.2 mL | 26.4 mL |

**Note** - the volume is sufficient for performing Cell Surface blocking, cocktail reconstitution, and washing with the 2-Wash protocol on page 11 of Next GEM Flex protocol CG000529 Rev C or page 12 of GEM-X Flex protocol CG000781 Rev A.

Next-GEM Flex - 10x Genomics Demonstrated Protocol for Cell Surface and Intracellular Protein Labeling for Fixed RNA CG000529 Rev C

GEM-X Flex - 10x Genomics Demonstrated Protocol for Cell Surface and Intracellular Protein Labeling for GEM-X Flex Gene Expression CG000781 Rev A

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### Reconstitution of the MultiPro® Human Discovery Panel

1. Determine the type of reaction pouch.

   Each MultiPro® Human Discovery Panel (HDP) reaction comes individually packaged with *either*:

   - One (1) Cell Surface (CS, purple cap) and two (2) Intracellular (IC, white cap) tubes **or**
   - One (1) Cell Surface (CS, purple cap) and one (1) Intracellular (IC, white cap) tubes

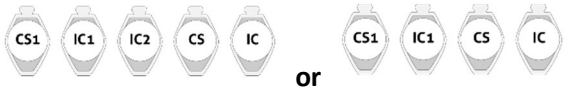
2. Remove the desired number of panel reactions from 4 °C storage.

   1 Reaction (RXN) of HDP


**or**

   **Critical:** All tubes in one reaction pouch are required to stain up to 2 million cells.

3. Label four or five 1.5 mL Protein LoBind tubes for reconstitution (depending on whether your HDP reaction contains 2 or 3 tubes; see above). Label one tube for each cocktail reaction component tube (CS1, IC1, IC2) and two for final collection (CS, IC). A sample layout is shown in below. *Without* removing the cocktail tube caps, equilibrate each tube to room temperature before proceeding with reconstitution.




**or**

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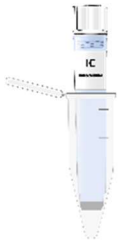
4. **Critical:** This step ensures that all lyophilized material is at the *bottom* of the tube prior to reconstitution. Steps 5 to 9 ensure the reconstituted cocktail is at the correct concentration.

   *Without* removing caps from the cocktail tubes, insert the cocktail tubes into unlabeled 1.5 mL microcentrifuge tubes and spin at 1500 x *g* for 30 seconds at RT.




5. Carefully reconstitute each lyophilized cocktail tube by adding the amount of BSA or IC Block and Staining Buffer indicated in the tables below.

   **Note** - if spiking-in antibodies to either the final CS or IC cocktails, you will have to adjust the amount of BSA Buffer or IC Block and Staining Buffer used in reconstitution of the individual HDP tubes as indicated below. For example, if spiking-in 1 µL of antibody to your CS tube, reconstitute the Cell Surface Cocktail tube with 51 µL BSA Buffer so that the total final volume will be 52 µL (spike-in + HDP).

   If your HDP pouch contains (1) Cell Surface (CS) and (1) Intracellular (IC) tube, use the following table for reconstitution:

| Component | Cap Color | Reconstitution Buffer | Buffer Volume |
|---|---|---|---|
| Cell Surface Cocktail | Purple | BSA Buffer | 55 µL |
| Intracellular Supplement | White | IC Block and Staining Buffer | 55 µL |

   If your HDP contains (1) Cell Surface (CS), (1) Intracellular (IC), *and* (1) IC Supplement tube, use the following table for reconstitution:

| Component | Cap Color | Reconstitution Buffer | Buffer Volume |
|---|---|---|---|
| Cell Surface Cocktail | Purple | BSA Buffer | 55 µL |
| Intracellular Cocktail | White | IC Block and Staining Buffer | 27.5 µL |
| Intracellular Supplement | White | IC Block and Staining Buffer | 27.5 µL |

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6. Re-cap the cocktail tubes and vortex for 15 seconds and spin at 10,000 x *g* for 30 seconds at RT.
7. Incubate for 5 minutes at RT.
8. Vortex the tubes for 15 seconds and spin at 10,000 x *g* for 30 seconds at RT.
9. Transfer the entire content of each reconstituted cocktail tube to its appropriately labeled Eppendorf tube. Purple cap to CS1, White cap(s) to IC1 and/or IC2.
10. **Critical:** This step pellets any protein aggregates that may be present in the reconstituted cocktail.

    Spin the Eppendorf tubes containing the combined, reconstituted cocktails at 14,000 x g for 10 minutes at 4 °C.
11. *Without* touching the bottom of the tube, use a pipette to carefully transfer the appropriate volume of reconstituted cocktail to the pre-labeled 1.5 mL Protein LoBind tubes from step 2, as indicated below:
    a. Transfer 52 µL of reconstituted CS cocktail from the CS1 tube to the CS labeled tube.
    b. Combine reconstituted IC cocktails IC1 and IC2 by transferring 26 µL of each into the labeled IC tube.
    c. Transfer 52 µL of reconstituted IC cocktails from IC1 into the labeled IC tube.
    d. If using multiple HDP reactions at a time, the tubes containing cocktails of the same type can be pooled together (e.g., pool all reconstituted CS cocktails into one tube and both the IC and IC Supplement combined in another tube).
12. Keep the reconstituted cocktails on ice until ready to use.
13. Proceed with the appropriate 10x Genomics protocol:

**Note** - Proteintech Genomics has validated the MultiPro® Human Discovery Panel using the 2-Wash option on page 11 of Next GEM protocol CG000529 Rev C or page 12 of GEM-X Flex protocol CG000781 Rev A.

Next GEM Flex: 10x Genomics Demonstrated Protocol for Cell Surface and Intracellular Protein Labeling for Fixed RNA CG000529 Rev C

GEM-X Flex: 10x Genomics Demonstrated Protocol for Cell Surface and Intracellular Protein Labeling for GEM-X Flex Gene Expression CG000781 Rev A

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**Critical:** If using GEM-X Flex, there are *required* changes to protocol CG000781 Rev A for optimal performance. The following changes *must* be implemented:

**Page 14, "Intracellular Protein Labeling"**

- Step b of "Intracellular Protein Labeling" is replaced by step 5 of this protocol. Steps have been recreated below and modified to show updated volumes.
- Step b. Add 55 µL IC Block and Staining Buffer to reconstitute lyophilized antibody panel and set aside the rest of the IC Block and Staining Buffer.
- **Note** - During panel rehydration and aggregate removal, one can proceed with the cell centrifugation step in ***step a.*** of ***Intracellular Protein Labeling: Label Samples*** section at page 17.
- Step h. Transfer 52 µL of supernatant to a new Protein LoBind tube and keep on ice. This is the Intracellular Antibody Mix Supernatant.

**Page 17, "Intracellular Protein Labeling: Label Samples"**

- Step c1. Prior to adding Intracellular Antibody Mix Supernatant, resuspend the cell pellet with ***50 µL*** IC Block and Staining Buffer, gently pipet mix 10 times and incubate for 10 mins at $4^0$C.
- Step c2. Add ***50 µL*** of Antibody Mix Supernatant to the blocked cells. Gently mix 10 times with pipet (set to 85 µl) and proceed to intracellular straining.

## Contact Us

Questions, concerns, or suggestions? Please contact us at **genomics.support@ptglab.com**