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%	EMS	15714
Human TruStain™ FcX	BioLegend	422302
1.5mL Protein LoBind tubes	Eppendorf	022431081

Required Equipment

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

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Before You Start

- Proteintech Genomics has validated the MultiPro® Human Discovery Panel with the following protocol:
    - 10x Genomics Demonstrated Protocol for Cell Surface and Intracellular Protein Labeling for Fixed RNA CG000529 (Rev C)

- **Time required -** Depending on the number of samples being stained, this protocol is expected to take ~3-4+ hours *after* cell preparation. The staining protocol *does not* contain any safe stopping points and users must *immediately* proceed to the "Sample Fixation" step of the "10x Genomics Fixation of Cells & Nuclei for Chromium Fixed RNA Profiling protocol (CG000478 Rev D)" and subsequent "Probe Hybridization" of the "Chromium Fixed RNA Profiling Reagent Kits for Multiplexed Samples with Feature Barcode technology for Protein using Barcode Oligo Capture protocol (CG000673 Rev A)", which requires ~2 hours of hands-on time.

- **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 30µm 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. Ascertain baseline antibody performance by performing cell fixation as described in "Cell Fixation" section of this document, followed by staining with fluor-conjugated antibodies of interest, and performing flow cytometry. Most of the antibodies in the MultiPro® Human Discovery Panel are available in fluorophore-conjugated versions at ptglab.com.

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- **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.

- **Sequencing Parameters**

For Cell Ranger v8.0 or newer
- Read 1: 28 cycles
- Index 1: 10 cycles
- Index 2: 10 cycles
- Read 2: 90 cycles

For Cell Ranger v7.2
- Read 1: 48 cycles
- Index 1: 10 cycles
- Index 2: 10 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 ultrapure water. Filter-sterilize using a 0.2 µm filter and store at 4 °C for up to one week.

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 Staining Buffer

Critical: **This buffer replaces "Antibody Buffer" in the "Intracellular Staining" section of the 10x Genomics demonstrated protocol.**

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	110.72
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	1x	17.1	68.4
FcX	-	5	20
10x PBS	1x	11	44
Nuclease-free Water	-	37.67	161.68
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
10X PBS	1x	0.330	1.32	2.64
Ultrapure 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 pg. 11 of the 10x Genomics Demonstrated Protocol for Cell Surface and Intracellular Protein Labeling for Fixed RNA CG000529 (Rev C)

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Reconstitution of the MultiPro® Human Discovery Panel

1. Each Multipro® Human Discovery Panel reaction comes individually packaged with (1) Cell Surface (CS, purple cap) and (2) Intracellular (IC, white cap) cocktail tubes. Remove desired number of panel reactions from 4 °C storage for use.

1 reaction of the MultiPro® Human Discovery Panel

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

2. Label five 1.5 mL Protein LoBind tubes for reconstitution, one for each cocktail reaction component tube (CS1, IC1, IC2) and two for final collection (CS, IC). A sample layout is shown in the image below. Without removing the cocktail tube caps, allow each antibody cocktail tube to sit at room temperature (RT) for 5 minutes.

3. Insert the cocktail tubes into unlabeled 1.5 mL microcentrifuge tubes and spin at 1500 x *g* for 30 seconds at RT.

Critical: Step 3 ensures that all lyophilized material is at the bottom of the tube prior to reconstitution. Steps 4 to 7 ensure the reconstituted cocktail is at the correct concentration.

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4. Carefully reconstitute each lyophilized cocktail tube by adding the amount of BSA or IC Staining Buffer indicated in the table below. Please note that if you are spiking-in antibodies into either the final CS or IC cocktails, you will have to adjust the amount of BSA Buffer or IC Staining Buffer used in reconstituting the individual tubes as indicated below:

Component	Cap Color	Reconstitution Buffer	Buffer Volume
Cell Surface Cocktail	Purple	BSA Buffer	52 µL
Intracellular Cocktail	White	IC Staining Buffer	55 µL
Intracellular Supplement	White	IC Staining Buffer	55 µL

Critical: **If you are spiking-in antibodies into the Multipro® Human Discovery Panel, the volume of reconstitution buffer used must be adjusted so that the total volume of reconstituted and individual antibodies equals the volumes as shown in the table above. For example, if adding 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.**

5. Re-cap the cocktail tube and vortex for 15 seconds and spin at 10,000 x *g* for 30 seconds at RT.

6. Incubate for 5 minutes at RT.

7. Vortex the tube for 15 seconds and spin at 10,000 x *g* for 30 seconds at RT.

8. Spin the Eppendorf tubes containing the combined reconstituted cocktails at 14,000 x *g* for 10 minutes at 4 °C.
Critical: **This step pellets any protein aggregates that may be present in the reconstituted cocktail.**

9. Without touching the bottom of the tube with a pipette tip, carefully transfer the appropriate volume of reconstituted cocktail to a new 1.5 mL Protein LoBind tube, as shown:

    - For pre-fixation staining – Transfer 50 µL of reconstituted CS cocktail from the CS1 tube to CS tube.
    - For post-fixation staining – Transfer 53 µL of reconstituted IC cocktails from IC1 and IC2 and combine into IC tube.

Critical: **Touching the bottom of the tube with a pipette tip or transferring the entire volume could result in the transfer of aggregates to the final staining solution.**

10. Keep reconstituted cocktail on ice until ready to use.

11. Proceed with:

    10x Genomics Demonstrated Protocol for Cell Surface and Intracellular Protein Labeling for Fixed RNA CG000529 (Rev C)

    Note: We have only validated Human Discovery Panel using the 2-Wash option on pg. 11 of the protocol (CG000529 Rev C).

Contact Us

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