[Deprecated] TotalSeq™-B or -C with 10x Feature Barcoding Technology V.2

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Abstract

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Buyer is solely responsible for determining whether Buyer has all intellectual property rights that are necessary for Buyer's intended uses of the BioLegend TotalSeq™ products. For example, for any technology platform Buyer uses with TotalSeq™, it is Buyer's sole responsibility to determine whether it has all necessary third party intellectual property rights to use that platform and TotalSeq™ with that platform.

The following protocol describes surface protein staining with TotalSeq™–B and TotalSeq™–C antibodies and/or hashtag antibodies, to enable protein detection in addition to Single Cell 3′ v3 and Single Cell V(D)J Feature Barcoding technology from 10x Genomics.

Please read the entire protocol before starting the experiments.

Materials

MATERIALS

Human TruStain FcX™ (Fc Receptor Blocking Solution) **BioLegend Catalog #**422301, 422302

Cell Staining Buffer **BioLegend Catalog #**420201

- Human TruStain FcX (Fc Receptor Blocking Solution, Cat. No. **422301**)
- Cell Staining Buffer (BioLegend Cat. No. **420201**)
- Dextran Sulfate Sodium Salt (MP Biomedicals, Cat. No. 101516 or equivalent)
- DNA LoBind Tubes (Eppendorf, Cat# 022431021)
- TotalSeq™–B antibodies and/or hashtag antibodies for Single Cell 3' v3 protocol with Feature Barcoding technology for Cell Surface Protein
- TotalSeq™–C antibodies and/or hashtag antibodies for Single Cell V(D)J protocol with Feature Barcoding technology for Cell Surface Protein
- Biotinylated antibody and oligo **barcoded** streptavidin

Troubleshooting

I) Sample and Solutions Preparation

1
- Prepare single cell suspension following a suitable protocol
- Prepare Dextran Sulfate solution: 1% w/v (10 mg/ml) Dextran Sulfate Sodium Salt in Nuclease-free Water

II) Cell labeling for 10x Genomics platforms

2 Carefully count all cells to ensure accurate quantitation.
- Make note of cell viability (>95%) and also include dead cells in the total cell count.
- If high cell death is observed, live cell enrichment (e.g. by Flow Cytometry) is recommended.

3 Resuspend 1–2 million cells in 50 µl Cell Staining Buffer.

4 3. Add 5 µl of Human TruStain FcX™ Fc Blocking reagent and 2 µl of Dextran Sulfate solution

5 4. Incubate for 10 minutes at 4°C.

6 5. While cells are incubating in Fc Block, prepare antibody pool using 1 µg (or titrated amounts) of each TotalSeq™ and/or hashtag or biotinylated antibody.

7 6. To maximize performance, centrifuge the antibody pool at 14,000xg at 2 – 8°C for 10 minutes before adding to the cells.
Note: If antibody cocktail volume is less than 50 µl, add Cell Staining Buffer up to 50 µl, then centrifuge

8 7. Carefully pipette out the liquid, avoiding the bottom of the tube, and add the TotalSeq™ antibody cocktail to the cell suspension.

9 8. Incubate for 30 minutes at 4°C.

10 9. Wash cells 3 times with 1 mL of Cell Staining Buffer, spin 5 minutes 350g at 4°C.
Note: It has been observed in some cases that various factors, including cell/sample type, tube manufacturer, rotor type, wash buffer, etc., may result in an excessive number of cells coating the side of the tube. Please ensure that staining and washing conditions are appropriate for your sample type.

11 10. If using biotinylated antibodies, incubate with the appropriate oligo **barcoded** streptavidin at the recommended amount specified in the product technical

datasheet for 20 minutes.

12 11. Wash cells 3 times with 1 mL of Cell Staining Buffer, spin 5 minutes 350g at 4°C.

13 12. Resuspend cells in PBS supplemented with 0.04% BSA.
Note: Based on starting cell concentration and assuming approximately 50% cell loss, calculate volume to achieve a final cell concentration of 700 – 1,200 cells/µl.

14 13. Filter cells through 40 µm strainers.

15 14. Verify cell concentration and viability by counting on hemocytometer after filtration.

- Chromium Single Cell 3' Reagent Kits v3 User Guide with Feature Barcoding technology for Cell Surface Protein (**CG000185 Rev B**) for TotalSeq–B reagents OR
- Chromium Single Cell V(D)J Reagent Kits User Guide with Feature Barcoding technology for Cell Surface Protein (**CG000186 Rev A**) for TotalSeq–C reagents