TotalSeq™ Universal Cocktails

Instructions for Use

The protocol below is intended for customers who are using TotalSeq™ Universal Cocktails.

Please read the entire protocol below and the appropriate 10x Genomics user guide before starting your experiments. The recommended 10x Genomics user guide for each TotalSeq™ Universal Cocktail is listed in the corresponding TotalSeq™-A, B, or C protocol. BioLegend protocols can be found at: biolegend.com/en-us/technical-protocols#proteogenomictotalseqprotocols

Table 1: Reconstitution and Staining Volumes

TotalSeq™ Format	Reconstitution Volume (µL)	No. of Cells	Volume of FcR Blocked Cells (µL)	Total Staining Volume (µL)
A, B, C	27.5	$5x10^5$	25	50

Custom cocktails may require different reconstitution instructions. Please reach out to your local BioLegend representative or distributor if you have questions regarding custom cocktails.

Lyophilized Panel Reconstitution and Staining

1. Equilibrate the lyophilized panel vial(s) to room temperature for 5 minutes.
2. Place lyophilized panel vial in an empty Eppendorf tube, spin down at 10,000 x *g* for 30 seconds at room temperature.
3. Rehydrate lyophilized panel by adding 27.5 µL of Cell Staining Buffer (BioLegend Cat. No. 420201).
   Replace the cap and vortex for 10 seconds.
   Note: Excess volume added to aid in removal of potential protein aggregates.
4. Incubate at room temperature for 5 minutes.
5. Vortex again and spin down at 10,000 x *g* for 30 seconds at room temperature.
6. Transfer the entire volume (27.5 µL) of reconstituted cocktail to a low protein binding Eppendorf tube (Fisher Cat. No. 022431081 or similar tube).
7. Centrifuge at 14,000 x *g* for 10 minutes at 4°C.
8. While cocktail is being centrifuged, resuspend and block cells. Perform staining in 12 x 75 mm tubes.
   a. For human cells add 2.5 µL of Human TruStain FcX™ Fc blocking reagent (BioLegend Cat. No. 422301) to 5 x 10^5 cells in 22.5 µL Cell Staining Buffer (total volume = 25 µL). Incubate for 10 minutes at 4°C.
   b. For mouse cells add 0.25 µL TruStain FcX™ PLUS (anti-mouse CD16/32) blocking reagent (BioLegend Cat. No. 156603) to 5 x 10^5 cells in 24.75 µL Cell Staining Buffer (total volume = 25 µL). Incubate for 10 minutes at 4°C.
9. Transfer 25 µL of reconstituted cocktail to the tube containing 25 µL of FcR blocked cells. The final staining volume is 50 µL.
10. Proceed with corresponding staining protocol:
    a. For TotalSeq™-A panels, start at step 8 of the following protocol:
    go.biolegend.com/totalseq-a-v3-protocol
    b. For TotalSeq™-B or TotalSeq™-C panels, start at step 8 of the following protocol:
    go.biolegend.com/totalseq-b-c-protocol