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 BioLegend protocol. BioLegend protocols can be found at: biolegend.com/en-us/technical-protocols#proteogenomictotalseqprotocols

Table 1: Applicable TotalSeq™ Universal Cocktails

TotalSeq™ Universal Cocktail Format	Cat. No.
TotalSeq™-A	399907
TotalSeq™-B	399904
TotalSeq™-C	399905

Table 2: Reconstitution and Staining Volumes

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

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 the recommended reconstitution volume (Table 2) 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 min at 4°C.
8. While cocktail is being centrifuged, block cells by adding 2.5 µL of Human TruStain FcX™ Fc blocking reagent to the recommended number of cells (Table 2) in 22.5 µL Cell Staining Buffer (total volume = 25 µL). Incubate for 10 min at 4°C. Perform staining in 12 x 75 mm tubes.
9. Transfer 25 µL of reconstituted cocktail to the tube containing 25 µL of FcR blocked cells (Table 2). 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