TotalSeq™-A Human Universal Cocktail v1.0

Instructions for Use

The protocol below is intended for customers who are using the TotalSeq™-A Human Universal Cocktail.

Please read the entire protocol below and the appropriate user guide for your single cell platform before starting your experiments. See Table 1 below for details.

Table 1: Reconstitution and Staining Volumes

Format	Species	Cat. No.	Vial Item No.	Reconstitution Volume (µL)	Reconstituted cocktail volume for staining (µL)	No. of cells	Volume of FcR blocked cells to stain (µL)	Total staining volume (µL)
TotalSeq™-A	Human	399907	750002094	27.5	25	$5x10^5$	25	50

Lyophilized Panel Reconstitution and Staining

1. Equilibrate the lyophilized panel vial(s) to room temperature for 5 minutes.
2. Spin down at 10,000 x *g* for 30 seconds at room temperature.
3. Resuspend lyophilized panel in recommended reconstitution volume (Table 1) of Cell Staining Buffer (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 1) in 22.5 µL Cell Staining Buffer (total volume = 25 µL). Incubate for 10 min at 4°C.
9. Transfer 25 µL of reconstituted cocktail to the tube containing 25 µL of FcR blocked cells (Table 1). Perform staining in 12 x 75 mm tubes. The final staining volume is 50 µL.
10. Proceed with staining by starting at step 8 of the TotalSeq™-A staining protocol: go.biolegend.com/staining-protocol