SIGNAL-seq: ADT Cell Staining Protocol v1.0

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Staining buffers: Scale buffer volumes for as many samples as necessary.

Wash Buffer:

Reagent	Stock Concentration	Final Concentration	Volume (µL)
BSA	5%	0.5%	100
Tween	10%	0.1%	10
Protectorase	40U/µL	0.1U/µL	2.5
Superase	20U/µL	0.1U/µL	5
PBS	NA	NA	882.5
Total Volume			**1000**

Blocking Buffer:

Reagent	Stock Concentration	Final Concentration	Volume (µL)
BSA	5%	1%	200
Tween	10%	0.1%	10
Protectorase	40U/µL	0.1U/µL	2.5
Superase	20U/µL	0.1U/µL	5
Dextran Sulphate	1%	0.025%	25
TruStain FcX	1x	100x	10
Sheared Salmon Sperm DNA	10 mg/mL	1 mg/mL	100
PBS	NA	NA	647.5
Total Volume			**1000**

Notes:

- Everything should be done on ice unless specified otherwise.
- The centrifuge should be pre-cooled to 4C before work.
- All pipetting involving cells should be done with wide-bore P200 or P1000 low retention pipette tips.
- Since the SPLiT-seq barcoding protocol commences immediately after this protocol it essential that the sample distribution map for the SPLiT-seq RT barcoding plate has already been calculated on a previous day.

Thaw Cell Samples and Stain with Oliog-ADT Conjugated Antibodies

1. Make the antibody cocktail first before starting as this can take a long time depending on the number of antibodies in the panel. Add any extra volume needed with Blocking Buffer and always add Rnase inhibitor cocktail.

2. Remove cells from the -80C freezer and place on ice. Thaw cell samples at 37C on a thermomixer until a small ice crystal remains and place samples on ice.

3. Add 200 µL (or less) Wash buffer to the tube to generate a total volume of 250 µL (plate well volume limit) depending on the initial volume of 0.5x PBS+DMSO in the tube.

4. Filter cells with a FlowMi 40 µm filter into 96-well plate

5. Spin 800 g 5 mins @ 4C and decant supernatant.

6. Add 100 µL of blocking buffer – incubate for 15 mins @ 4C.

8. Spin 800 g 5 mins @ 4C and decant supernatant.

9. Resuspend by 10x pipetting up and down gently with a wide-bore pipette in 25 µL blocking buffer with pipette set to 15 µL.

10. Add 25 µL staining cocktail to each sample and incubate for 30 mins @ RT, with gentle pipetting set to 30 µL at every 10 mins.

11. Resuspend in 200 µL blocking buffer and spin 800 g 5 mins @ 4C and decant supernatant.

12. Repeat **step 11** with 200 µL of blocking buffer.

13. Repeat wash **step 11** with 200 µL of wash buffer.

14. Resuspend in appropriate volume of 0.5x PBS + RI (depends on cell input number).

15. Filter 1-3x through a 40 µm FLOWMI filter and into new wells of the 96-well plate.

16. Take 5/10 µL of volume and count with Trypan using Countess, check the level of doublets there should be effectively none in the cell suspension. If there are cell doublets of clumps then re-filter through a 40 µm FLOWMI filter.

17. Check there are no clumps in the cell mixture – otherwise filter a subsequent time with FLOWMI filter and count again.

18. Adjust cell concentration to desired concentration using 0.5x PBS + RI to achieve the correct cell concentration for loading into the SPLiT-seq reverse transcription plate.

NB: It helps to layout your samples in a similar format as they will be applied in the 48 well RT barcoding plate. The cells can then be easily transferred into the SPLiT-seq RT plate at the beginning of the SPLiT-seq barcoding protocol.

Proceed directly to SIGNAL-seq: Split-pool Barcoding Protocol