WU sc-prep Protocol for Solid Tumors v2.1

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1 Works for me
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ABSTRACT

Single-cell Isolation for solid tumors Ding Lab 2021

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Reagents

1 -Human tumor dissociation kit(Miltenyi Biotec #130-095-929)
-PBS
-PBS, 0.5% BSA (keep at 4°C)
-(optional) Red Blood Cell (RBC) Lysis Solution ACK buffer Fisher (A1049201)
-Falcon 15mL/50mL Conical Centrifuge Tubes (Corning Life Sciences DL, Fisher Scientific)
-Large filter: Fisherbrand Cell Strainers. Nylon mesh 40um (Fischer Scientific, Cat # 22-363-547)
-Smaller filter: PluriSelect Mini-Strainer 40 uM (Cat #43-10040-60)
-Petri dishes
-LoBind Protein or Genomic Microcentrifuge Tubes, Eppendorf®, Volume=2 ml Fisher 13-698-792

-All steps should be performed under sterile conditions
-For tissue fragments >1000mg, scale up reagent volumes (volumes listed are for 1 g tissue)

Octo Dissociation

2 Mince tissue into small pieces with razor blade in petri dish.

3 Resuspend tissue in enzyme mix (2.5 mL DMEM to 100 uL of Enzyme H, 50 uL Enzyme R, and 12.5 uL enzyme A) in C-Tube

4 Incubate on gentleMACS™ Octo Dissociator - 37h_TDK_1 setting (incubation can be shortened if tissue is very soft ~20-25 min). Check sample every 10-15 minutes.

5 After dissociation, filter cells through 40um filter and spin down in 15ml tube at 450g for 5 min at 4 degrees

6 If there is any red visible in the cell pellet proceed to ACK RBC lysis step below (Refer to bottom of protocol for examples).
RBC ACK Lysis Solution
-Slowly resuspend cell pellet in 500 uL-5mL (5ml for larger pellets) of RBC ACK Lysis Solution
-Incubate on ice for 30 sec to 5 min (longer incubation for more RBCs)
-Quench reaction with 10mL of BSA/PBS buffer
-Centrifuge at 450g 5min at 4 degrees

7 Do 1-3 1mL washes of 0.5% BSA in PBS on ice (spin at 450g 5min at 4 degrees) to remove dead cells and debris. Do washes in 2mL tube. After each wash aspirate off BSA/PBS and leave ~50-100 uL of BSA/PBS buffer. Resuspend cells using 1000 uL wide-bore pipette tip and count 10 uL of sample using a hematocytometer and trypan blue.

After first wash take 10 uL of sample and visualize under microscope (for smaller amounts take 2 uL of sample and 8 uL of wash buffer). If after 1 wash there are too many dead cells – consider using dead cell removal kit or debris removal kit:

- Dead Cell Removal: https://www.miltenyibiotec.com/US-en/products/macs-sample-preparation/removal-reagents/dead-cell-removal-kit.html
- Debris Removal: https://www.miltenyibiotec.com/US-en/products/macs-sample-preparation/removal-reagents/debris-removal-solution.html

oNote: For Debris removal – use slow speed 450-500 g for all spinning steps.
If cell suspension looks pretty clean after first wash, then second wash should be clean to load.
oIf you have approximately 400-2000 cells/uL then continue on to GEMs if doing single cell. Load 20,000 cells.

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