FEB 25, 2021

🌐 WU sc-prep Protocol for Solid Tumors v2.1

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ABSTRACT

Single-cell Isolation for solid tumors Ding Lab 2021

OPEN ACCESS

DOI:

dx.doi.org/10.17504/protocols.io.bsnqnddw

Protocol Citation: Reyka Jayasinghe, Li Ding, Feng Chen 2021. WU sc-prep Protocol for Solid Tumors v2.1.

protocols.io

<https://dx.doi.org/10.17504/protocols.io.bsnqnddw>

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Protocol status: Working
We use this protocol and it's working

Created: Feb 22, 2021

Last Modified: Feb 25, 2021

PROTOCOL integer ID:
47536

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 hemacytometer 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>

Note: 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.

If you have approximately 400-2000 cells/uL then continue on to GEMs if doing single cell. Load 20,000 cells.