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# WU sn-prep Protocol for Solid Tumors - snRNA protocol v2.8

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

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## Abstract

**WU sn-prep Protocol for Solid Tumors -snRNA protocol v2.7**

## Troubleshooting

## Reagents and Tools

1

1x Lysis buffer (2mL):
10mM Tris-HCl (pH 7.4) (Thermo; 15567027), 20 uL
10mM NaCl (Thermo; AM9759), 4 uL
3 mM MgCl2 (Thermo; AM9530G), 6 uL
NP-40 substitute (Sigma, 74385-1L), 2 uL
1 M DTT (Sigma, 646563), 2 uL
Nuclease Free Water (Invitrogen, AM9937), 1.966 mL

2 Lysis Dilution Buffer (10 mL):
10mM Tris-HCl (pH 7.4) (Thermo; 15567027), 100 uL
10mM NaCl (Thermo; AM9759), 20 uL
3 mM MgCl2 (Thermo; AM9530G), 30 uL
1 M DTT (Sigma, 646563), 10 uL
Nuclease Free Water (Invitrogen, AM9937), 9.840 mL

3 0.1x Lysis Buffer (10 mL):
1x Lysis Buffer (1mL) + Lysis Dilution Buffer (9 mL)

4 Wash and Resuspension buffer (WR buffer):
1X PBS, 8 mL
10% Stock BSA Solution (MACS, 130-091-376), 2 mL

5 Storage buffer:
1X PBS +2% BSA + 10% glycerol (for freezing) + 0.2U/ul RNase inhibitor
Hard to pipette 100% glycerol. Please make 60% glycerol in 1X PBS. Do a 1:2 mix with the Wash and Resuspension buffer.

6 Trypan blue (2X) - filtered at 0.22 mm.

7 7-AAD (7-Aminoactinomycin D) (millipore sigma SML1633-1ML)

8 Glass homogenizers (Fisher: 2mL tube- K8853030002, Small clearance pestle- K8853020002, Large clearance pestle- K8853010002)

9 Fine forceps and scalpels

## General Notes

10
- Keep everything on ice (or in the cold room).
- Use RNase free reagents and consumables (Use filtered tips).
- Avoid foam and bubbles as much as possible by gentle strokes and pipetting.
- For filtering step, remember to backwash the filter and examine the stuff blocked by the filter if necessary

## Single Nucleus Prep for sn-Seq

11 If using frozen tissue sample, use a scalpel (aided by a pair of fine forceps) to cut the cold samples (25-35mg) into 2mm pieces. Load the pieces into the glass homogenizer. Homogenize by 4-6 pushes and 4-6 pulls using the pestle in a glass homogenizer in 1 ml of ice-cold 0.1X lysis buffer with 30 uL (40 U/ul) RNase Inhibitor. Incubate on ice for 1 min with an additional 1 ml of 0.1X cold lysis buffer. Pipette gently for 4 times. Incubate on ice again for up to 1 min.

12 If using pulverized powder, start with 15-35 mg total. Pipette the powder/lysis buffer mix gently for 6-8 times. Let sit on ice for 30″. Pipette another 4-6 times. Incubate on ice again for 1′ – could be reduced (to like 20-45″). You may choose to homogenize via pestle for 3-4 push/pulls.

13 Filter the homogenate through a 40mm cell strainer on ice. Wash the filter with 1ml wash buffer. Collect this into the same filtrate, so the total filtrate is 3 ml. If there is still tissue on the strainer, backwash with 2 mL 0.1x Lysis buffer, and follow previous steps again. If going to FACS with the backwash, proceed with this sample as if it were a different tissue and then sort into same collection tube.

14 Transfer this to a 5ml Eppendorf tube. Centrifuge at 500g for 6′ at 4°C and resuspend in up to 400ul Wash buffer + ~10 uL RNase inhibitor.

15 Transfer into a FACS tube and add 1 uL 7-AAD per 500 uL of sample, and incubate for 10 minutes before FACS. After resuspending sample in wash buffer, if small chunks are still visible (after ~3 minutes of resuspension) use 40 uM mini-strainer over FACS tube to remove chunks (proceeding to FACS with sample in current condition will clog machine and will result in additional lost sample).

16 Add 50 uL WR buffer into a 2 mL nonbinding tube for collection with 10 uL RNAse inhibitor. Collect ~100K nuclei into collection tube.

17 Once FACS is done, centrifuge at 500g for 6′ at 4°C, remove supernatant but leave ~40 uL left in tube. Resuspend in additional WR buffer + RNase inhibitor as needed if too concentrated. Count and check nuclei quality under the microscope and prepare for loading according to the 10X protocol.