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FixNCut v1.0

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1 more workspace ↓



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ABSTRACT

This protocol details reversible fixation for cells and tissues for subsequent use in sc/snRNA, sc/snATAC or Multiome. Spatial-Omics compatibility is being validated. For more information check this preprint: https://www.biorxiv.org/content/10.1101/2023.06.16.545221v2

ATTACHMENTS

748-1909.docx

Notes:

All washes and centrifugations need to be done at [4 ° C unless otherwise specified.

IMPORTANT: Washing volumes may change accordingly to your needs. If you want to change the protocol, let's discuss just in case. Time, temperatures and concentrations must be maintained.

DSP has been used before for fixing cells and prep RNA, it works fine. For single cell or tissue following dissociation is what we have been studying and works well. It's still work in progress, but the key is to keep the stock fixative away from water because it neutralizes the NHS-esters quickly.

Points to take into account:

- **CRITICAL**: Prepare working solution (1x) right before fixation (no more than 5 minutes). For larger pieces replace with fresh 1x fixative a couple of times.
- make single use aliquots (20-50 uL) for 2 to 5 fixations. What's not used do not re-freeze (it is fine to re freeze let's not give them the option)
- keep aliquots at 8 -80 °C in a bag (with silica if possible).
- bring tubes at Room temperature and prepare the fixation a few minutes (no longer than 10 min) before fixing. This will ensure that the NHS-ester isn't in contact with aqueous solution for too long.
- evaluate small precipitation during fixation. Too much ppt: bad. You should see a small ppt on the walls of the tube, like in the attached photo. You will notice that the first 2 drops of PBS will be generate the precipitate but will precipitate as you add more.
- do not store 1x solutions.
- We have noticed some performance variability from vial to vial purchased from Sigma.
- Viability is not a good measure because PI or Trypan Blue don't enter after fixation and the cells look alive.
- So far, the best test has been the small shift in the LMO-FAM or LMO-Cy5 on cells.

MATERIALS

Liberase™ TM Research Grade Merck MilliporeSigma (Sigma-Aldrich) Catalog
#5401127001

Nuclei Isolation Kit: Nuclei EZ Prep Merck MilliporeSigma (Sigma-Aldrich) Catalog #NUC1011KT

Preparation of DSP (Oz Soup) stock and working solutions

Note

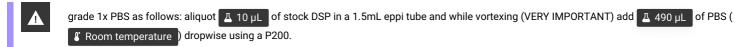
DSP (dithiobis(succinimidyl propionate)) also known as Lomant's Reagent and can be purchased from Thermo:
 https://www.thermofisher.com/order/catalog/product/22585.

DSP (dithiobis(succinimidyl propionate)) Lomants Reagent Thermo Fisher Scientific Catalog #22585

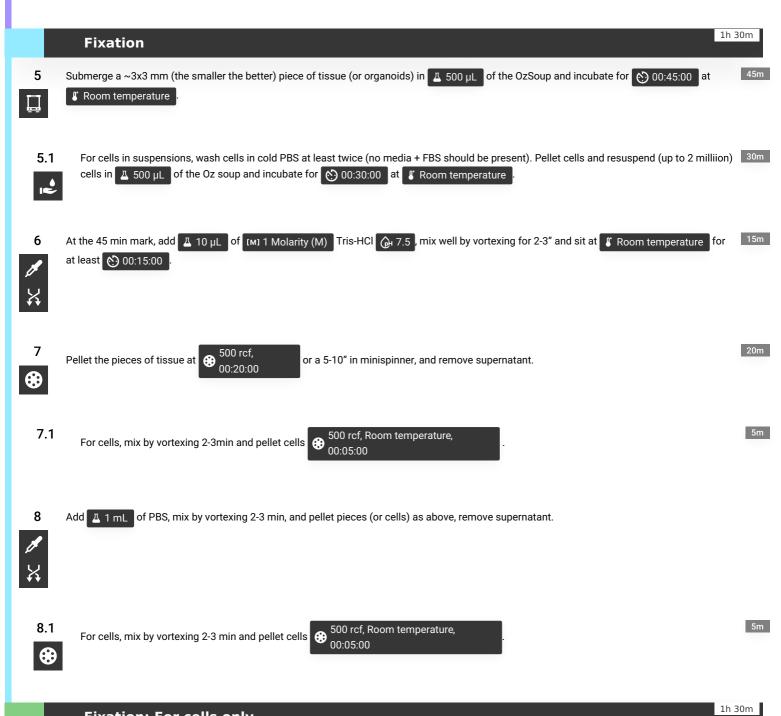
Equilibrate DSP vial at Room temperature for 00:30:30:00 and then prepare 50x stock solution of DSP 50 mg/mL in molecular biology grade dimethyl sulfoxide (Sigma, cat. no. D8418-50ML).

Dispense the stock into 100 µL aliquots and store at 8.80 °C.

Immediately before use prepare 50x stock solution (DSP 1x is also known as OzSoup) in molecular biology

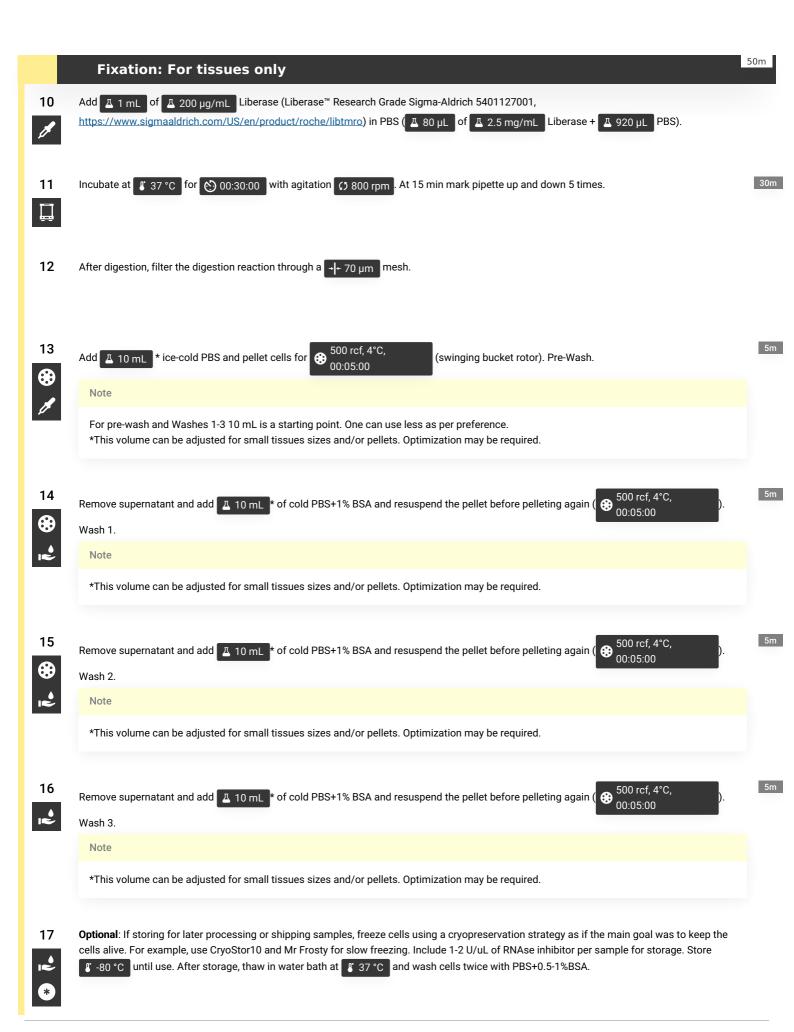


4 Filter DSP working solution using a 40-μm Flowmi strainer (Sigma, cat. no. BAH136800040-50EA).



Fixation: For cells only

9 Repeat 8 once more for a total of 2 washes. Continue on step 18-21 below. If sorting or shipping samples follow step 17.



18	Remove supernatant and resuspend cells in	n 🔼 0.5-1 mL of PBS+1% BSA (optionally add +0.5-1 U/uL RNAse Inhibitor).
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- 19 Filter cells through Flowmi 40 um.
- 20 Count cells and bring concentration to 1000-1500 cells/uL.
- 21 Load Chromium as per manual.

Note

For ATAC or Multiome kits prpware nuclei using EzLysis Buffer (Sigma-Aldrich, Cat: NUC101-1KT), SaltyEz10/50 protocols (dx.doi.org/10.17504/protocols.io.bx64prgw) or alternatives you are familiar with.