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DSP-Methanol Fixation for Cells

Demonstrated Protocol

ILLUMINA PROPRIETARY
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Introduction

These protocol instructions describe best practices for cell fixation prior to input into Illumina Single Cell 3’ RNA Prep kits. This cell fixation protocol uses a mixture of DSP (dithiobis(succinimidyl propionate)) and methanol for both primary (eg, PBMC) and cultured cell lines (eg, HEK/3T3).

The Illumina Single Cell Prep Support page on the Illumina support site provides additional training material.

Consumables & Equipment

The protocol requires the following user-supplied consumables and equipment.

Consumables

Consumable	Supplier
1 M Tris-HCl pH 7.5	Teknova, catalog # T5075
10% Bovine Serum Albumin (BSA)	Sigma-Aldrich, catalog # A1595
100% Anhydrous DMSO	Thermo Fisher, catalog # D12345
Absolute methanol (MeOH)	Sigma-Aldrich, catalog # 34860
Cell counting materials (eg, AO/PI)	General lab supplier
Cell Suspension Buffer (CSB)	Included in all Illumina Single Cell Prep kits
Conical tube, 15 ml, sterile and nuclease-free	General lab supplier
DEPC-Treated Water (µl)	Thermo Fisher, CAT#AM9906
DTT (1 M)	Sigma-Aldrich, catalog # 646563
[Optional] DTT powder, solid	Sigma-Aldrich, catalog # D9779, or equivalent
Low retention microcentrifuge tube, 1.5 ml	General lab supplier
Nuclease-free water	Thermo Fisher, catalog # AM9937, or equivalent
NxGen RNase inhibitor (40 U/µl)	Biosearch Technologies, catalog # 30281-1
Pierce DSP, No-Weigh Format	Thermo Fisher, catalog # A35393
Pipette tips, filtered, low retention, sterilized, 20 µl	One of the following suppliers: • Rainin, catalog # 30389226 • VWR, catalog # 89031-350 • Filtrous, catalog # PTF-LS-0020
Pipette tips, filtered, low retention, sterilized, 200 µl	One of the following suppliers: • Rainin, catalog # 30389240 • VWR, catalog # 89031-372 • Filtrous, catalog # PTF-LS-0200
Pipette tips, filtered, low retention, sterilized, 1000 µl	One of the following suppliers: • Rainin, catalog # 30389213 • VWR, catalog # 89031-412 • Filtrous, catalog # PTF-LS-1000

Consumable	Supplier
Pipette tips, wide-orifice, 200 µl	Rainin, catalog # 30389241
Saline sodium citrate (SSC) (20X)	Sigma-Aldrich, catalog # S6639

Equipment

Equipment	Supplier
Benchtop microcentrifuge 500 × g, suitable for 1.5 ml tubes and 15 ml conical tubes	General lab supplier
Hemocytometer or automated cell counter	General lab supplier
Ice bucket or cold blocks, suitable for 15 ml conical tubes and 1.5 ml microcentrifuge tubes	General lab supplier
Micropipettes, 1 µl–1000 µl capabilities (Rainin LTS-tip compatible pipettes are required if you are using the recommended Rainin pipette tips)	General lab supplier

Protocol

This section provides step-by-step instructions on how to preserve samples using DSP-Methanol fixation. Before proceeding, make sure that you have the necessary consumables and equipment. Refer to *Consumables & Equipment* on page 2.

Buffer Preparation

This section describes buffer preparation for an input of 1 million cells in a 200 µl volume. Optimize the volume based on specific cell numbers, sample numbers, or Cell Suspension Buffer (CSB) volume.

Consumables

- Absolute methanol (MeOH)
- DMSO
- DSP
- 1.5 ml low retention microcentrifuge tube
- 1000 µl pipette tips

Procedure

Fixative Preparation (Prepare Fresh)

1. Prepare the DSP solution as follows.
   a. Dissolve DSP into DMSO at 50 mg/ml concentration.
   For example, dissolve 1 mg of DSP in 20 µl 100% Anhydrous DMSO to generate 20 µl of DSP solution.

      One 20 µl aliquot of DSP solution can fix up to 10^6 cells.

   b. Vortex to mix, and then centrifuge briefly.
2. In a 1.5 ml tube, combine the following volumes to prepare a working solution of fixative.
   - DSP solution (20 µl)
   - Chilled 100% MeOH (800 µl)
3. Using a 1000 µl pipette, gently pipette to mix.
4. Keep the fixative on ice until use.

DSP-Methanol Fixation

Consumables

- 1 M Tris-HCl pH 7.5
- 10% Bovine Serum Albumin (BSA)
- Absolute methanol (MeOH)
- Cell Suspension Buffer (CSB)
- DTT (1 M)
- **[Optional]** DTT powder, solid
- Low retention microcentrifuge tube, 1.5 ml
- Nuclease-free water
- NxGen RNase inhibitor (40 U/µl)
- Pierce DSP, No-Weigh Format
- Pipette tips, wide-orifice, 200 µl
- Saline sodium citrate (SSC) (20X)

Fixation Procedure

1. For each cell type of interest, resuspend up to 1 million cells in 200 µl CSB, then leave on ice. During cell counting, make sure to note the cell viability for later calculations.
2. Slowly add the chilled fixative (~820 µl) to the 1.5 ml tube containing the sample. Swirl while adding the fixative.
3. Allow the cells to fix on ice for 30 minutes. Gently swirl occasionally to mix the solution.
4. Add 20.4 µl 1M Tris pH 7.5.
5. Using a wide-orifice 200 µl pipette tip, gently pipette to mix.
6. Store fixed cells at -25°C to -15°C for up to one week.

Post-Fixation Resuspension

1. Prepare the Resuspension Buffer immediately before use.

   1 M DTT can be purchased or made using solid DTT powder and nuclease-free water.

   Table 1 Resuspension Buffer (based on 1200 µl total buffer volume)

Reagent	Initial Concentration	Final Concentration	Volume to add to Master Mix (µl)
SSC	20X	3X	180
DTT	1000 (mM)	1 (mM)	1.2

Reagent	Initial Concentration	Final Concentration	Volume to add to Master Mix (µl)
RNase Inhibitor	40 (U/µl)	0.2 (U/µl)	6
BSA	10 (% by mass)	1 (% by mass)	120
Nuclease-free water	Not applicable	Not applicable	892.8

2. Remove the fixed cells from -25°C to -15°C storage and equilibrate on ice to 4°C.
3. Centrifuge at 500 × g for 5 minutes, preferably at 4°C.
4. Carefully remove supernatant and discard properly.

   This waste contains methanol and DSP. Be sure to discard in accordance with the applicable standards for your region.

5. While resuspending, assume a loss of 40–50% of input cells. Use chilled Resuspension Buffer to resuspend the cell pellet and achieve the appropriate cell concentration based on the planned scale of your Illumina Single Cell 3' RNA Prep kit.

   For 1 million cells, a final resuspension volume of ~100 µl should result in a concentration of ~5000 cells/µl, which is optimal for the T20 format. If the cell concentration is lower than desired, introduce an additional centrifugation step to adjust the resuspension volume.

6. Determine cell concentration using your preferred method (eg, AO/PI staining on an automated cell counter). Dilute the cells to the desired working concentration with Resuspension Buffer. Leave everything on ice.
   If you observe clumping, prepare a fresh Resuspension Buffer using fresh DTT and repeat steps 3–5 to resuspend the cells.

   In general, methanol-fixed cells appear dead in live/dead dual-fluorescence stainings (eg, AO/PI), however the DSP might complicate this readout. To arrive at a reasonable working cell count, Illumina recommends that you take the total cell count (instead of live or dead) and multiply it by the previously noted initial viability.

Resources & References

The Illumina Single Cell Prep Support page on the Illumina support site provides additional resources. These resources include training, compatible products, and other considerations. Always check the support pages for the latest versions.

Revision History

Document	Date	Description of Change
Document # 200067419 v01	January 2026	Corrected measurements in the table used for preparing the Resuspension Buffer in the post-fixation resuspension. Updated recommendations for user-supplied consumables.
Document # 200067419 v00	March 2025	Initial release.

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