

DSP-Methanol Fixation for Nuclei

Demonstrated Protocol

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Introduction

These protocol instructions describe best practices for nuclei fixation prior to input into Illumina Single Cell 3' RNA Prep kits. This nuclei fixation protocol uses a mixture of DSP (dithiobis(succinimidyl propionate)) and methanol.

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
100% Anhydrous DMSO	Thermo Fisher Scientific, catalog # D12345
25% BSA	Included in all Illumina Single Cell Prep kits
Absolute methanol (MeOH)	Sigma-Aldrich, catalog # 34860
Cell counting materials (eg, AO/PI)	General lab supplier
Conical tube, 5 ml, sterile and nuclease-free	General lab supplier
Low retention microcentrifuge tube, 1.5 ml	General lab supplier
Nuclease-free water	Thermo Fisher, catalog # AM9937 or general lab supplier
Nuclei Suspension Buffer (NSB)	Included in all Illumina Single Cell Prep kits
Pierce DSP, No-Weigh Format	Thermo Fisher Scientific, catalog # A35393
Pipette tips, filtered, low retention, sterilized, 20 µl	One of the following suppliers: • Rainin, catalog # 30389226 • VWR; 76322-528
Pipette tips, filtered, low retention, sterilized, 200 µl	One of the following suppliers: • Rainin, catalog # 30389240 • VWR, catalog # 76322-150
Pipette tips, filtered, low retention, sterilized, 1000 µl	One of the following suppliers: • Rainin, catalog # 30389213 • VWR, catalog # 76322-154
Pipette tips, wide bore, 200 µl	Customer provided
RNAse Inhibitor (RNI)	Included in all Illumina Single Cell Prep kits

Equipment

Equipment	Supplier
Benchtop microcentrifuge 500 x 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](#).

Prepare for Protocol

1. Combine the following volumes to prepare the NSB working solution.

- Nuclei Suspension Buffer (167 μ l)
- Nuclease-free water (833 μ l)

This prepares 1 ml of NSB working solution. Scale up or down based on requirement.

2. Combine the following volumes to prepare the NSB-BSA-RNI working solution.

- Nuclei Suspension Buffer (167 μ l)
- 25% BSA (40 μ l)
- RNI (20 μ l)
- Nuclease-free water (773 μ l)

This prepares 1 ml of NSB-BSA-RNI working solution. Scale up or down based on requirement.

Buffer Preparation

This section describes buffer preparation for an input of 1 million nuclei in a 200 μ l volume. Optimize the volume based on specific nuclei numbers, sample numbers, or Nuclei Suspension Buffer (NSB) volume.

Consumables

- Absolute methanol (MeOH)
- DMSO
- DSP
- 1.5 ml low retention microcentrifuge tube
- 1000 μ l 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 μ l 100% Anhydrous DMSO to generate 20 μ l of DSP solution.

i | One 20 μ l aliquot of DSP solution is capable of fixing up to 10^6 nuclei.

- 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 μ l)
 - Chilled 100% MeOH (800 μ l)
3. Using a 1000 μ l pipette, gently pipette to mix.
4. Keep the fixative on ice until use.

DSP-Methanol Fixation

Consumables

- 1 M Tris-HCl pH 7.5
- Conical tube, 5 ml, sterile and nuclease-free
- Nuclease-free water
- Nuclei Suspension Buffer (NSB)
- Pierce DSP, No-Weigh Format
- Pipette tips, filtered, low retention, sterilized, 1000 μ l

Fixation Procedure

1. For each nuclei tissue type of interest, resuspend up to 1 million nuclei in 200 μ l NSB working solution in a 5 ml tube and leave on ice.
2. Slowly add the chilled fixative (~820 μ l) to the 5 ml tube containing the sample. Swirl while adding the fixative.
3. Allow the nuclei to fix on ice for 15 minutes. Gently swirl or tap every 3–4 minutes to mix the solution.
4. For each 1 ml of fixation sample, add 20.4 μ l 1M Tris pH 7.5.
5. Using a 1000 μ l pipette, gently pipette to mix.
6. Store fixed nuclei at -25°C to -15°C for up to 2 months.

Post-Fixation Resuspension

1. Remove the fixed nuclei from -25°C to -15°C storage and equilibrate on ice to 4°C .
2. For each 1 ml of nuclei in fixative, add 2 ml of NSB working solution gradually. Swirl between additions.

3. Centrifuge at 500 x g for 3 minutes, preferably at 4°C.
4. Carefully remove supernatant and discard properly.
The nuclei pellet is at the bottom and appears off-white in color.

 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 nuclei. Use chilled NSB working solution to resuspend the nuclei pellet and achieve the appropriate concentration based on the planned scale of your Illumina Single Cell 3' RNA Prep kit. Triturate gently with a narrow-bore P200 µl pipette tip to separate nuclei and avoid aggregates.

For 1 million nuclei a final resuspension volume of ~100 µl should result in a concentration of ~5000 nuclei/µl, which is optimal for the T20 format. If the nuclei concentration is lower than desired, introduce an additional centrifugation step to adjust the resuspension volume. Refer to the following table for target nuclei concentration for the respective Illumina Single Cell 3' RNA Prep kit.

Kit Size	Starting Nuclei	Desired Concentration (nuclei/µl)	Post-Fixation Resuspension volume (µl NSB working solution)
T2	1,000,000	1,250	400
T10	1,000,000	3,400	147
T20	1,000,000	5,000	100
T100	1,000,000	10,000	50

6. Determine nuclei concentration using your preferred method (eg, AO/PI staining on an automated cell counter). Dilute the nuclei to the desired working concentration with the prepared NSB-BSA-RNI working solution. Leave everything on ice.

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 # 200067424 v01	September 2025	Added separate Prepare for Protocol section to cover the preparation of working solutions. Added table with target nuclei concentrations for different Illumina Single Cell 3' RNA Prep kit sizes. Clarified that Rainin LTS-tip compatible pipettes are required if you are using the recommended Rainin pipette tips. Renamed working solutions for additional clarity.
Document # 200067424 v00	March 2025	Initial release.



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