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ACME-sorbitol (ACMEsorb) Fixation of Cells in Suspension

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We use this protocol and it's working

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Abstract

ACME (Acetic-Methanol) maceration is a tissue dissociation and fixation method described by Jordi Solana's group that preserves both cell morphology and RNA integrity, and is compatible with single-cell transcriptomics (scRNA-seq) (García-Castro et al., 2021). ACME has been tested on various organisms, including freshwater planarians (*Dugesia japonica*), annelids (*Pristina leidyi*), snails (*Limnaea stagnalis*), vertebrate embryos (*Mus musculus* and *Danio rerio*), terrestrial arthropods (*Parasteatoda tepidariorum* embryos and *Drosophila melanogaster* larvae), and the starlet sea anemone (*Nematostella vectensis*), which inhabits brackish waters (García-Castro et al., 2021).
However, for marine organisms, this protocol proved suboptimal in our hands, as we observed cells bursting immediately upon contact with the fixative solution—presumably due to the hypo-osmolarity of the fixative relative to seawater. To compensate for the osmolarity and preserve cell integrity during fixation, we replaced the water (or 1× PBS) fraction in the original ACME formulation with a 0.8 M sorbitol solution (Najle et al., 2023). This modified version, which we named **ACMEsorb**, was successfully applied for single-cell atlasing of various marine species. The protocol version presented here is optimized for the fixation of dissociated cells derived from marine organisms that either do not easily dissociate mechanically after fixation with ACMEsorb (like for example, placozoans) or those possessing abundant ECM material (like jellyfish or ctenophores).

Guidelines

If your tissue or organism of interest dissociates easily and the cells do not tend to clump after centrifugation, you can proceed with Protocol A. If you prefer to prevent clumping before fixing your sample, or if your cells are fragile, proceed with Protocol B.

Materials

Stock Solutions:

2.4 M Sorbitol

- Weigh 218.6 g of sorbitol (Sigma-Aldrich #S1876) and place it in a 1 L container.
- Add 250 mL of nuclease-free water (NFW) and dissolve using a magnetic stirrer. Sorbitol dissolution is endothermic, so gentle heating may be required to aid dissolution.
- If some crystals remain undissolved, add a small amount of additional water, being careful not to exceed a total volume of 500 mL.
- Once fully dissolved, use a volumetric cylinder to bring the volume up to 500 mL with NFW.
- Filter the solution through a 0.22 µm membrane using a bottle-top vacuum filter system (e.g., Corning CLS430517).
- Store at room temperature.

10% BSA (Nuclease-Free)

- Add 10 mL of NFW to a bottle of BSA Fraction V (Sigma-Aldrich #126609-10GM).
- Dissolve thoroughly by gentle agitation, minimizing foam formation.
- Dispense into 1 mL aliquots and store at –20 °C.

Calcium and magnesium-free artificial seawater (CMFSW)

- Weigh the following reagents and place in a clean 1 L recipient:
  0.18 g of $NaHCO_3$ (Sigma-Aldrich #S5671)
  0.8 g of KCl (Sigma-Aldrich #P9541)
  27 g of NaCl (Sigma-Aldrich #S7653)
  1 g of Na_2SO_4 (Sigma-Aldrich #239313)
  10 mL of Tris-HCl pH8 (Life Technologies #15568-025)
- Add Milli-Q water to approximatelly 800 mL and stir using a magnetic stirrer until all salts are fully dissolved.
- Once fully dissolved, use a volumetric cylinder to bring the volume up to 1 L with Milli-Q water.
- Filter the solution through a 0.22 µm membrane using a bottle-top vacuum filter system (e.g., Corning CLS430517).
- Store at room temperature.

Resuspension Buffer 1 (RB1)

- Prepare 1X calcium- and magnesium-free PBS (Ca-Mg-free PBS) as the buffer base.
- Add 10% BSA (Nuclease-free) to a final concentration of 0.1%. For example, add 100 µL per 1 mL of final volume.
- Add sorbitol to a final concentration of 0.8 M. For example, if using a 2.4 M sorbitol stock, add 333 µL per 1 mL of final volume.
- Add RiboLock RNase inhibitor (Thermo Fisher # EO0382) to a final concentration of 0.2 U/µL (e.g., 5 µL of RiboLock per 1 mL of buffer).
- Mix gently by inversion or pipetting. Avoid vortexing to preserve enzyme activity.
- Prepare fresh or keep on ice until use.

Other reagents:

- Methanol (Sigma Aldrich #34860-1L-R)
- Glacial Acetic Acid (Merck # 1000631000)
- Glycerol (Sigma Aldrich # G7757-1GA)
- 10X Ca-Mg-free PBS (Thermo Fisher AM9624)

Safety warnings

- Use **ultrapure reagents** (free of nucleases), and keep your pipettes and workbench clean and free of RNases. Clean surfaces with **RNaseZap** or a similar product throughout the entire experiment.
- Use **nuclease-free water** (e.g., Sigma-Aldrich #W4502-1L) for all solutions and buffers.
- During sample preparation, keep all reagents and samples on **ice**, unless otherwise specified in the protocol.
- **ACMEsorb-fixed cells** are highly resistant to mechanical stress. This is why a high centrifugation speed (2500 × *g*) is used after fixation—to maximize cell recovery. However, this speed can be **reduced** to minimize cell clumping, provided that clumping is observed after thoroughly resuspending the pellet.
- **DO NOT** add ACMEsorb fixative directly to a cell **pellet**, as this will lead to the formation and fixation of clumps that are very difficult to dissociate later.

Before start

Prepare an appropriate volume of **ACMEsorb solution** and **Resuspension Buffer 1 (RB1)** based on the number of samples to be processed. Refer to the 'Materials' section for the composition of each solution.

Begin with a (mostly) monodisperse cell suspension, prepared with your preferred dissociation method—such as dissociation in calcium- and magnesium-free artificial seawater (CMFSW), buffers containing cation-chelating agents like EDTA, or enzymatic tissue digestion.

The quality of the cell suspension should be assessed microscopically and adjusted as needed for each specific system. Aim to balance between thorough dissociation and a cell viability >85%. Ideally, avoid proceeding with a cell suspension containing less than 85% viable cells.

Protocol A

1h 5m

1 After pelleting cells at the minimum speed necessary for recovery, gently re-suspend the pellet i 50 µL of [M] 1.2 Molarity (M) sorbitol. 2m

2 Immediately add 1 mL of freshly prepared ACMEsorb mix of the following composition: 5m

- 650 µl 1.2M sorbitol
- 100 µl glycerol
- 100 µl glacial acetic acid
- 150 µl methanol

3 Gently pipette up and down twice to mix, and incubate for 00:40:00 at Room temperature on a rotary shaker set at 25 rpm . 40m

4 After fixation is completed, pellet the cells by 5m
2500 x g, 4°C, 00:05:00 , in a swing-bucket rotor.

5 Carefully remove **3/4 volume** (750 µL) of ACMEsorb solution, without disturbing the cell pellet. 2m

6 Add 750 µL of Resuspension Buffer 1 (RB1), of the following composition: 2m

- 1X Ca-Mg-free PBS
- 0.8M sorbitol
- 0.1% nuclease-free BSA
- 0.02 U/µl RiboLock

7 2500 x g, 4°C, 00:05:00 5m

8 Remove the supernatant and re-suspend the pellet in 900 µL RB1. 2m

9 Add 100 µL of DMSO, invert three times to mix and freeze at -80 °C in a Nalgene® Mr 2m

Frosty freezing container following the recommendations of the manufacturer.

Note that no filtration step through cell strainer is performed before freezing the samples. This is to avoid sample loss at this stage, as it will be performed before FACS-sorting (YES, WE STRONGLY RECOMMEND FACS-sorting YOUR SINGLE CELLS!).

Protocol B

1h 5m

10 Once dissociation is complete (assuming dissociation was performed in a total volume of 1 mL CMFSW-based buffer), transfer cell suspension to a 5 ml protein LoBind tube. 2m

11 Slowly add the following ACMEsorb premix to the cell suspension to complete 3 mL : 5m

- 950 µl 1.2M sorbitol
- 300 µl glycerol
- 300 µl glacial acetic acid
- 450 µl methanol

12 Invert twice to mix and incubate for 00:40:00 , at Room temperature in a rotary shaker set at 25 rpm . 40m

13 Pellet fixed cells by 2500 x g, 4°C, 00:05:00 using a swing-bucket rotor. 5m

14 Carefully **remove 3/4 volume** (2000 µL) of ACME solution, without disturbing the cell pellet. 2m

15 Add 2000 µL of Resuspension Buffer 1 (RB1), of the following composition: 2m

- 1X Ca-Mg-free PBS
- 0.8M sorbitol
- 0.1% nuclease-free BSA
- 0.02 U/µl RiboLock

16 2500 x g, 4°C, 00:05:00 5m

17 Remove the supernatant and re-suspend the pellet in 900 µL RB1. 2m

18 Add 100 µL of DMSO, invert three times to mix and freeze at -80 °C in a Nalgene® Mr Frosty freezing container following the recommendations of the manufacturer. 2m

Note that no filtration step through cell strainer is performed before freezing the samples. This is to avoid sample loss at this stage, as it will be performed before FACS-sorting (YES, WE STRONGLY RECOMMEND FACS-sorting YOUR SINGLE CELLS!).

Protocol references

1: García-Castro H, Kenny NJ, Iglesias M, Álvarez-Campos P, Mason V, Elek A, Schönauer A, Sleight VA, Neiro J, Aboobaker A, Permanyer J, Irimia M, Sebé-Pedrós A, Solana J. ACME dissociation: a versatile cell fixation-dissociation method for single-cell transcriptomics. Genome Biol. 2021 Apr 8;22(1):89. doi:10.1186/s13059-021-02302-5. PMID: 33827654; PMCID: PMC8028764.

2: Najle SR, Grau-Bové X, Elek A, Navarrete C, Cianferoni D, Chiva C, Cañas-Armenteros D, Mallabiabarrena A, Kamm K, Sabidó E, Gruber-Vodicka H, Schierwater B, Serrano L, Sebé-Pedrós A. Stepwise emergence of the neuronal gene expression program in early animal evolution. Cell. 2023 Oct 12;186(21):4676-4693.e29. doi:10.1016/j.cell.2023.08.027. Epub 2023 Sep 19. PMID: 37729907; PMCID:PMC10580291.