Sep 24, 2025

ACME-sorbitol (ACMEsorb) Fixation and Enzymatic Dissociation of Whole Soft-Bodied Cnidarians

DOI
https://dx.doi.org/10.17504/protocols.io.14egnr83ql5d/v1

Sebastián Najle[1], Léa-Lou imparé[1], Arnau Sebe-Pedros[1,2]

[1]Center for Genomic Regulation (CRG); [2]ICREA

Biodiversity Cell Atlas Methods Development Workspace
Tech. support email: **sebastian.najle@crg.eu**

Sebastián R. Najle
Center for Genomic Regulation (CRG)

OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.14egnr83ql5d/v1

Protocol Citation: Sebastián Najle, Léa-Lou imparé, Arnau Sebe-Pedros 2025. ACME-sorbitol (ACMEsorb) Fixation and Enzymatic Dissociation of Whole Soft-Bodied Cnidarians. **protocols.io** https://dx.doi.org/10.17504/protocols.io.14egnr83ql5d/v1

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

Created: July 15, 2025

Last Modified: September 29, 2025

Protocol Integer ID: 222499

Keywords: BCA_method, scRNA-seq, ACMEsorb, cold protease, dissociation, acmesorb fixation of whole organism, marine organism, cell atlasing of various marine species, starlet sea anemone, various marine species, bodied cnidarians acme, cell integrity during fixation, various organism, cell morphology, whole organism, vertebrate embryo, drosophila melanogaster larvae, rna integrity, including freshwater planarian, cell integrity, freshwater planarian, cell, nematostella vectensi, cell dissociation, cell transcriptomic, mechanical dissociation after acmesorb fixation, acmesorb fixation, embryo, snail, terrestrial arthropod, annelid, seawater, parasteatoda tepidariorum embryo, fixation with acmesorb, short fixation with acmesorb, enzymatic dissociation of whole soft, cell atlasing, enzymatic dissociation, osmolarity of the fixative relative

Funders Acknowledgements:
Gordon & Betty Moore Foundation
Grant ID: GBMF12189

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. ACMEsorb fixation of whole organisms, followed by cell dissociation, is the preferred method, as it minimizes cellular stress and reduces viability biases observed during the dissociation of fresh samples.

Dissociation post-fixation can be performed either enzymatically, as described here, or mechanically (a protocol using mechanical dissociation after ACMEsorb fixation is available [here]). In this protocol, we use cold-protease to dissociate whole specimens of the starlet sea anemone *N. vectensis*, after a short fixation with ACMEsorb.

Guidelines

- Use ultrapure reagents (free of nucleases) and keep your pipettes and workbench clean and free of RNases (clean surfaces with RNase Zap or similar) throughout the whole experiment.
- Use nuclease-free water (for example, Sigma-Aldrich #W4502-1L) for all the solutions and buffers. During sample prep, keep all the reagents and samples on ice unless otherwise indicated in the protocols.
- ACMEsorb-fixed cells are very resistant to mechanical stress. This is the reason for the high centrifugation speed (1500 x g) used after fixation, which is intended to maximize cell recovery. However, this speed can be optimized (reduced) to avoid cell clumping, provided this is observed after thoroughly resuspending the pellet.

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 nuclease-free water 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.

Nematostella vectensis Calcium and magnesium-free artificial seawater (Nv-CMFSW)

- Weigh the following reagents and place in a clean 1 L recipient:
  0.06 g of $NaHCO_3$ (Sigma-Aldrich #S5671)
  0.26 g of KCl (Sigma-Aldrich #P9541)
  9 g of NaCl (Sigma-Aldrich #S7653)
  0.33 g of Na_2SO_4 (Sigma-Aldrich #239313)
  3.33 mL of 1M 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)
- 1M $CaCl_2$ (Thermo Fisher #J63122.AE)
- Cold protease (Sigma-Aldrich #P5380). Dissolve to 125 mg/mL with nuclease-free water, aliquot and freeze at -20 °C

Safety warnings

Volumes of fixative solution and buffers are orientative and should be adjusted according to the amount of material. As a reference, the volumes specified here were used for 3 *N. vectensis* specimens of ~1cm each.

Ethics statement

The protocols.io team notes that research involving animals and humans must be conducted according to internationally-accepted standards and should always have prior approval from an Institutional Ethics Committee or Board.

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.

Procedure

1h 40m

1 Transfer animal(s) to a small Petri dish or 6-well plate, in a minimum volume of artificial seawater (ASW) at the corresponding salinity depending on the species. Here, we use 14 ppm (1.4 g/L) Red Sea Salts for *N. vectensis* — 5m

2 Quickly rinse the animals twice with 2 mL Nv-Calcium and Magnesium-free seawater (Nv-CMFSW) — 5m

3 Remove as much Nv-CMFSW as possible, and add 3 mL of freshly made ACMEsorb solution of the following composition: — 3m
- 1950 µl 1.2M sorbitol
- 300 µl glycerol
- 300 µl glacial acetic acid
- 450 µl methanol

4 Immediately chop the animals into 3-4 mm pieces using a scalpel or a razor blade — 3m

5 Using a wide bore tip or a plastic Pasteur pipette, transfer the tissue pìeces in ACMEsorb to a 5 ml protein LoBind tube — 1m

6 Gently invert the tube 3 times to mix, and incubate for 00:15:00 at Room temperature on a rotary shaker set at 25 rpm — 15m

7 Once fixation is completed, collect the fixed tissue pieces by 1500 x g, 4°C, 00:05:00 , in a swing-bucket rotor — 5m

8 Remove (2.7 mL) supernatant, leaving ~300 µl in the tube and resuspend the pellet in that volume — 2m

9 Add 1 mL of Resuspension Buffer 1 (RB1), supplemented with 1 mM $CaCl_2$ — 2m

10 Repeat centrifugation at 1500 x g, 4°C, 00:05:00 , in a swing-bucket rotor — 5m

11 Carefully remove as much supernatant as possible and resuspend the pellet with 1 mL RB1 with [M] 1 millimolar (mM) $CaCl_2$ 2m

12 Add 40 µL [M] 125 mg/mL cold protease (Sigma-Aldrich #P5380) 2m

13 Incubate On ice , pipetting every ~2 minutes, for a total time of 00:15:00 15m

14 Quench the reaction by adding 1 mL of RB1 supplemented with [M] 10 millimolar (mM) EDTA. Invert twice to mix 3m

15 Spin down by 1500 x g, 4°C, 00:05:00 , in a swing-bucket rotor 5m

16 Carefully remove supernatant and resuspend the pellet with 1 mL RB1 3m

17 Filter through a 40 µm cell strainer into a new 1.5 mL protein LoBind tube 2m

18 Count cells using a Neubauer chamber on a 1:100 dilution of the cell suspension stained with [M] 1 µg/µL DAPI 10m

19 Prepare aliquots of ~1×10^6 cells in 900 µL RB1 10m

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

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.