

Nuclei isolation from flash-frozen *Pleurobrachia* samples

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Last update: Nov 5th, 2025

– PROTOCOL –

Materials

- | | |
|--|---|
| <input type="checkbox"/> BSA 1 % | <input type="checkbox"/> Nuclease-free water |
| <input type="checkbox"/> Buffer, lysis (stock) | <input type="checkbox"/> PBS for single cell |
| <input type="checkbox"/> Buffer, wash (stock) | <input type="checkbox"/> Petri dish |
| <input type="checkbox"/> Cell strainer 100 µm | <input type="checkbox"/> Pipettes |
| <input type="checkbox"/> Cell strainer Flowmi® 40 µm | <input type="checkbox"/> RNase inhibitor |
| <input type="checkbox"/> Dissecting needles | <input type="checkbox"/> RNaseZap™ |
| <input type="checkbox"/> Dounce homogenizers & pestels | <input type="checkbox"/> Samples |
| <input type="checkbox"/> DTT | <input type="checkbox"/> Scaple blades (new) |
| <input type="checkbox"/> Eppendorfs 0.5 mL | <input type="checkbox"/> Tips 1000 µL |
| <input type="checkbox"/> Eppendorfs 2 mL | <input type="checkbox"/> Tips 1000 µL low retention |
| <input type="checkbox"/> Eppendorfs 2 mL low bind | <input type="checkbox"/> Tips 200 µL |
| <input type="checkbox"/> Eppendorfs 5 mL | <input type="checkbox"/> Tips 200 µL low retention |
| <input type="checkbox"/> Falcon tubes 15 mL | <input type="checkbox"/> Tips 20 µL |
| <input type="checkbox"/> Gloves | <input type="checkbox"/> Tips 10 µL |
| <input type="checkbox"/> Ice boxes | <input type="checkbox"/> Tweezers |

Procedure

Make sure to set the temperature of the 4 °C centrifuge beforehand.

*Always **work on ice**, unless otherwise specified.*

*Always keep the working environment and materials as much **RNase-free** as possible.*

Place all the working solutions, tubes, and homogenizers on ice before starting.

Keep the frozen samples at -80 °C until use. Otherwise, place them in dry ice.

1. Add **2–5 mL of ice-cold working lysis buffer** into a Dounce homogenizer.
2. Transfer the samples from the tube to the homogenizers using tweezers.

If the starting material is too much, place it on a dry-ice-cooled petri dish, and split it in multiple samples using a sterile scalpel blade.

3. Homogenize samples on ice, with a maximum of 10 loose-pestel strokes, and a maximum of 10 tight-pestle strokes.
4. Rinse the pestles back into the homogenizers with **100–500 µL of lysis buffer**.

If the sample does not fully dissociate, incubate on ice with the lysis buffer for a couple of minutes.

5. Filter the homogenate through a 100 µm cell strainer into a 15 mL Falcon tube, using 1000 µL low retention tips.
6. Rinse the cell strainer with **1× PBS for single cell**.
7. Filter the homogenate through a 40 µm Flowmi® cell strainer into a clean 15 mL Falcon tube, using 1000 µL low retention tips.
8. Spin samples at 500–900 g for 5 min at 4 °C.
9. Discard the supernatant.
10. Resuspend the nuclei with **2 mL of working nuclei wash buffer**, gently pipetting with 1000 µL low retention tips.
11. Repeat steps 8–10 twice.
12. Resuspend samples in **500 µL of 1× PBS for the single-nuclei workflow**.

– RECIPES –**Stock lysis buffer**

Compound	Quantity	Initial concentration	Final concentration (for 10 mL)
Tris-HCl (pH 7.5)	100.0 µL	1.0 M	10.0 mM
NaCl	20.0 µL	5.0 M	10.0 mM
MgCl ₂	30.0 µL	1.0 M	3.0 mM
BSA	1.3 mL	7.5 %	1.0 %
IGEPAL® CA-630	100.0 µL	10.0 %	0.1 %
Nuclease-free water	8,416.0 mL	–	–

1. Add Tris-HCl (pH 7.5), NaCl, and MgCl₂ to combin;
2. add IGEPAL® CA-630 and BSA, mixing gently to avoid foaming;
3. add nuclease-free water to reach a final volume of 10 mL;
4. filter sterilise with a 0.2 µm filter;
5. store at 4 °C for 3–4 weeks, or at –20 °C for up to 6 months.

Avoid repeated freeze-thaw cycles. Thaw on ice. Mix gently to avoid foaming.

Working lysis buffer

Compound	Quantity	Initial concentration	Final concentration (for 1000 µL)
Dithiothreitol (DTT)	10.0 µL	0.1 M	0.0 M
RNAse inhibitor	5.0 µL	40.0 U/µL	0.2 U/µL
Stock lysis buffer	985.0 µL	–	–

1. Add DTT and RNAse inhibitor to the stock lysis buffer.

Stock wash buffer

Compound	Quantity	Initial concentration	Final concentration (for 25 mL)
Tris-HCl (pH 7.5)	250.0 µL	1.0 M	10.0 mM
NaCl	50.0 µL	5.0 M	10.0 mM
MgCl ₂	75.0 µL	1.0 M	3.0 mM
BSA	3.3 mL	7.5 %	1.0 %
Nuclease-free water	21.3 mL	–	–

1. Add Tris-HCl (pH 7.5), NaCl, and MgCl₂ to combin;
2. add BSA, mixing gently;
3. add nuclease-free water to reach a final volume of 25 mL;
4. filter sterilise with a 0.2 µm filter;
5. store at 4 °C for 3–4 weeks, or at –20 °C for up to 6 months.

Avoid repeated freeze-thaw cycles. Thaw on ice. Mix gently to avoid foaming.

Working wash buffer

Compound	Quantity	Initial concentration	Final concentration (for 1000 μL)
Dithiothreitol (DTT)	10.0 μ L	0.1 M	0.0 M
RNAse inhibitor	5.0 μ L	40.0 U/ μ L	0.2 U/ μ L
Stock wash buffer	985.0 μ L	–	–

1. Add DTT and RNAse inhibitor to the stock wash buffer.