Nuclei isolation from flash-frozen *Pleurobrachia* samples

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– PROTOCOL –

Materials

- ☐ BSA 7.5 %
- ☐ Buffer, lysis (stock)
- ☐ Buffer, wash (stock)
- ☐ Cell strainer 100 µm
- ☐ Cell strainer Flowmi® 40 µm
- ☐ Dissecting needles
- ☐ Dounce homogenizers & pestels
- ☐ DTT
- ☐ Eppendorfs 0.5 mL
- ☐ Eppendorfs 2 mL
- ☐ Eppendorfs 2 mL low bind
- ☐ Eppendorfs 5 mL
- ☐ Falcon tubes 15 mL
- ☐ Gloves
- ☐ Ice boxes
- ☐ Nuclease-free water
- ☐ Petri dish
- ☐ Pipettes
- ☐ RNase inhibitor
- ☐ RNaseZap™
- ☐ IMS
- ☐ Samples
- ☐ Surgical scalpel blades (sterile)
- ☐ Tips 1000 µL
- ☐ Tips 1000 µL low retention
- ☐ Tips 200 µL
- ☐ Tips 200 µL low retention
- ☐ Tips 20 µL
- ☐ Tips 10 µL
- ☐ Trypan Blue, 0.4 % solution
- ☐ 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; keep in ice.
2. Place the sample onto the lid of a 5 mL eppendorf tube filled with 500–1000 µL of pre-chilled lysis buffer.
3. Using a surgical sterile scalpel blade, **cut the sample into small chunks**.
4. Transfer the samples from the tube to the homogenizers using tweezers and/or a pipette, depending on chunk sizes.
5. **Homogenize samples on ice**, with a maximum of 10 loose-pestel strokes, and a maximum of 10 tight-pestle strokes.
6. Let big pieces of tissues to settle at the bottom of the homogenizer.
7. Repeat homogenization if the tissue does not look dissociated.

> *Always rinse the pestles back into the homogenizers with **100–500 µL of lysis buffer**.*

8. **Filter the homogenate** through a 30–40 µm cell strainer into a 5 mL eppendorf tube, using 1000 µL low retention tips.
9. Rinse the cell strainer with nuclei lysis buffer.
10. **Spin samples** at 500 g for 10 min at 4 °C.
11. Discard the supernatant and leave ~50 µL of buffer.
12. Add **600 µL of nuclei wash buffer** drop-wise without disturbing the pellet.
13. **Resuspend the nuclei** by gently pipetting with a 200 µL low retention tip, making sure they stay close to the bottom of the tube.
14. **Spin samples** at 500 g for 10 min at 4 °C.
15. Discard the supernatant and leave ~25 µL of buffer.
16. Add **50 µL of wash buffer** and resuspend the pellet by gently pipetting.
17. Possibly filter the nuclei suspension through a 10 µm cell strainer to avoid aggregates.

– RECIPES –

Working 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 %
Dithiothreitol (DTT)	10.0 µL	1.0 M	1.0 mM
RNAse inhibitor	50.0 µL	40.0 U/µL	0.2 U/µL
Protease inhibitor (#15614189)	1.0 tablet	–	–
Nuclease-free water	8,380.0 mL	–	–

1. Add Tris-HCl (pH 7.5), NaCl, and $MgCl_2$ to combin;
2. add IGEPAL® CA-630 and BSA, mixing gently to avoid foaming;
3. add RNAse inhibitor;
4. add 4 mL of nuclease-free water;
5. add the protease inhibitor tablet and let dissolve, also by gently pipetting;
6. add the remaining amount of nuclease-free water;
7. filter sterilise with a 0.2 µm filter;
8. use same day.

Working nuclei wash 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 %
Dithiothreitol (DTT)	10.0 µL	1.0 M	1.0 mM
RNAse inhibitor	50.0 µL	40.0 U/µL	0.2 U/µL
Nuclease-free water	8,457.0 mL	–	–

1. Add Tris-HCl (pH 7.5), NaCl, and $MgCl_2$ to combin;
2. add BSA, mixing gently;
3. add nuclease-free water to reach a final volume of 10 mL;
4. filter sterilise with a 0.2 µm filter;
5. use same day.