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Nuclei extraction from Drosophila embryos V.1

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

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Abstract

A simple protocol for the efficient Drosophila embryo nuclei extraction.

Materials

PBTX buffer (for 10 ml):

9.9 mL 1x PBS buffer

100 µL of 10% Triton-X solution (Triton + MQ water)

HB buffer (for 10 ml):

	Additive	Volume / Mass
	Sucrose (dry)	1.164 g
	1 M Tris-HCl pH 7.4	150 µL
	5 M NaCl	30 µL
	1 M KCl	600 µL
	0.5 M EDTA	4 µL
	0.5 M EGTA	4 µL
	Milli-Q water	add to 10 mL total (≈ 8.212 mL, accounting for the liquids above)

Add a protease inhibitor cocktail tablet, rotate until it dissolves, then filter through 0.2 Filtropur S membrane

PBTB buffer (for 10 ml):

Add 5% BSA to 10 mL of PBTX, i.e. 0.5 g . Rotate until BSA dissolves, add a protease inhibitor tablet, rotate until it dissolves, filter through the 0.2 Filtropur S membrane.

1 Collect the aged embryos from your juice plates. Use brush and mesh filter. 2m

2 Dechorionate the embryos in the bleach for 00:05:00 to 00:10:00 10m

3 Wash the embryos in the distilled water, check for the complete dechorionation under the microscope. 5m

4 Transfer the embryos to the 1.5 ml Eppendorf tube on ice, add 1 mL of PBTX, wait for embryos to precipitate, remove the supernatant, wash with PBTX 2 more times. 10m

5 Remove supernatant, add 1 ml of HB buffer, transfer embryos in HB buffer to the small dounce homogenizer on ice. Leave for 5 minutes on ice. 5m

6 Dounce **20** times with the **loose** pestle. 1m

7 Dounce **10** times with the **tight** pestle. 1m

8 Prepare two layers of Miracloth. They should cover a small glass funnel which should be compatible with 1.5 ml Eppendorf tube. The Miracloth layers should be rotated by 90° to each other's grain and placed into the funnel. Wash the cloth with the distilled water. 3m

9 Use a truncated (with scissors) 200 µl tip on top of the 1 ml tip to collect the nuclei extract from the dounce homogenizer. Filter the lysate through the two Miracloth layers into the 1.5 ml tube. Squeeze the cloth gently to drain the lysate. It's okay to get a larger volume than 1 mL .

10 Spin at 2000 g on 4 °C for 00:05:00 to pellet the nuclei. Remove the murky supernatant. 5m

11 (Optional) Wash the nuclei in 1 mL of HB buffer. Pellet them by spinning at 2000 g on 4 °C for 00:05:00 . It's possible to lose a lot of nuclei at this step. 5m

12 Remove the supernatant and add 500 µL of freshly made PBTB buffer. Prepare a 1 ml syringe and a 19-G needle and a 22-G needle. 1m

13 Dissociate the nuclei by passing them **8 times** through a **19-G** needle and then **8 times** through a **22-G** needle. 5m

13.1 **IMPORTANT!!!**
Before inserting the syringe into the tube, move the plunger a couple of times. Not doing so may result in a sudden aspiration of all the nuclei with the air creating bubbles which may disrupt the nuclei integrity.

14 Cut the 1 ml pipette tip with scissors. Collect the nuclei with this tip. Wrap a 47 mm 20 µm Nylon Net Millipore filter **securely** around it and expel the nuclei solution into a new 1.5 ml tube.

15 Count your nuclei with a counting chamber, taking 1-5 µL of your sample and mixing it with Trypan Blue solution.