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Immunofluorescence staining on larval and adult Drosophila gonads V.1

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

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Abstract

Immunofluorescence staining protocol for *Drosophila* gonads

Materials

1x PBS
0.3% PBTx (0.3% Triton-X in PBS)
1% PBTx (1% Triton-X in PBS)
Paraformaldehyde or formaldehyde
Normal serum (usually NGS)
Primary antibodies
Secondary antibodies
DAPI

Before start

All steps are done with gentle rotation.

Day 1

2h 40m

1 Dissect tissue in 1x PBS. Transfer to a 1.5 mL tube containing **1x PBS**. If it is a quick dissection (<20 mins), no ice needed. If more time is needed, keep samples on ice.

2 Remove PBS and add fixative. Fix in 4% paraformaldehyde in **0.3% PBTx** for 20 min RT with gentle rotation. 20m

500 uL fixative = 125 uL of 16% paraformaldehyde + 375 uL 0.3% PBTx

3 Aspirate the fixative and wash twice for 10 min in **1% PBTx**. Not getting rid of fix will affect your immunostaining. 20m

4 Aspirate the supernatant and block/permeabilize for at least 2 hours in **1% PBTx** + 5% normal serum, or overnight at 4°C. 2h

1 mL block solution = 50 uL NGS + 950 uL 1% PBTx

5 Primary antibodies are diluted in **0.3% PBTx** + 5% normal serum and incubate for 1 hour at RT or overnight at 4°C. Overnight will give better staining. 12h

Primary antibody mix in 0.3% PBTx + 15 uL NGS (300 uL total)

Day 2

5h 20m

6 Remove the primary antibody mix and wash in **0.3% PBTx** three times for 20 min at RT. 1h

7 Wash in **0.3% PBTx** + 5% normal serum twice for 30 min at RT. 1h

1 mL wash solution = 50 uL NGS + 950 uL 0.3% PBTx

8 Secondary antibodies are diluted in **0.3% PBTx** + 5% normal serum and incubated for ~2 hours at RT or overnight at 4°C. Keep tubes covered from light. 2h

9 Aspirate the supernatant and add 500 uL DAPI to each tube. Incubate for 10 min at RT. Keep tubes covered from light. 10m

10 Aspirate the supernatant and wash in **0.3% PBTx** three times for 20 min at RT. 1h

11 Aspirate the supernatant and wash in **PBS** for 10 min at RT. 10m

12 Store in **PBS** at 4°C or proceed with mounting. Keep tubes covered from light.

Protocol references

Slaidina et al. (2020)