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Fixation and Immunostaining protocol

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Protocol status: Working

We use this protocol and it's working

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Abstract

Fixation and Immunostaining protocol for tissue and cell culture

Materials

Buffers

2x Fix: 8% PFA in 2xPBS: Prepare from

32%PFA in sealed EMS ampule, + 8ml 10x PBS, + 22ml ddH₂O, stable light protected at 4 degrees for 2 weeks.

Fix: prepare fresh on day of use. Mix 2x Fix with ddH₂O, 1:1

Quench: Wash Buffer + 100mM Glycine + 0.1%

Sodium Azide

Block: Wash Buffer + 10% normal donkey serum + 0.1% Sodium Azide

Wash: 1x PBS + 0.1% Triton-X 100

DAPI 50x stock: dilute -80 frozen DAPI

aliquot (50Kx concentrate) 1:1000 in

filtered TC grade H₂O; store LP, 4°C up to 4 months



- 1 Prepare 1x Fix by dilution from 2x Fix (see Description)
- 2 Gently aspirate culture supernatant, wash gently with PBS, and replace with cold Fix buffer
- 3 Incubate 30min @ 4° C or on ice 30m
- 4 Aspirate fix and wash gently 3x with PBS (incubate 1-2min each)
- 5 Aspirate and add Quench to permeabilize cells/tissue, at least 15 min room temperature (RT). Note: this and all steps in humidified chamber (HC). Can store samples for weeks/months at 4 degrees
- 6 Aspirate and add Block buffer, 30 min RT 30m
- 7 Incubate with primary antibodies cocktail (1-2 hrs RT or 4°C overnight (ON))
- 8 Wash 3x 5min (wash buffer) 15m
- 9 Prepare secondary antibodies in Wash buffer (1:500-1:1000), filter, light protect (LP).Note: This and all subsequent steps LP 17h
- 10 Incubate with secondary antibodies 1hr RT, light protected (LP) 1h
- 11 Wash 3× 5min 15m
- 12 Incubate with 1x DAPI in Wash buffer 5-10 min 10m
- 13 Wash 1x



- 14 Rinse very briefly with filtered H₂O, 2x
- 15 Remove excess water, do not dry samples
- 16 Add Fluoromount-G (~20ul/well or ~100ul/slide) and apply No 1.5H coverslip (slowly lower, no bubbles) or other mountant
- 17 Dry ON, LP, RT