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Fixation and Immunostaining protocol

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gustavo.parfitt Parfitt[1], Gist Croft[2]

[1]University College of London; [2]NYSCF

ASAP Collaborative Rese… | Ahfeldt Lab

gustavo.parfitt Parfitt
University College of London

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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 ddH20, stable light protected at 4 degrees for 2 weeks.
Fix: prepare fresh on day of use. Mix 2x Fix with ddH20, 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 H_2O; 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 3x 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