

Immunocytochemistry

Section of Cancer Genomics, Genetics Branch, NCI
National Institutes of Health

Reagents

1X Phosphate Buffered Saline (PBS)

Bovine Serum Albumin (BSA)

Boehringer Mannheim, Cat. 100 350

DAPI

Sigma, Cat. D9542

EGTA

Sigma, Cat. E4378

HEPES

ICN Biomedicals, Inc., Cat. 101926

MgCl₂

Quality Biochem., Inc., Cat. 340-034-060

Mouse anti-gamma tubulin

Sigma, Cat. T6557

Normal Goat Serum (NGS)

Sigma, Cat. G6767

PIPES

Sigma, Cat. P8658

Sheep anti-mouse-FITC

Sigma, Cat. F3008

Water, sterile

Preparation

PHEM Buffer

PIPES	1.81	g	f.c. [60 mM]
HEPES	0.06	g	f.c. [25 mM]
0.5M EGTA	20	ml	f.c. [10 mM]
2M MgCl ₂	1	ml	f.c. [2 mM]
Water, sterile	978	ml	

*pH to 6.9 with approximately 900 μ l 10M NaOH

0.5M EGTA

EGTA 3.804 g

Water, sterile 20 ml

*pH to 7.5 to get EGTA into solution

Procedure

1. Wash cells (in chambers if using chamber slides) 2x with 1X PBS
2. Carefully permeabilize cells (in chambers if using chamber slides) with 0.5% Triton in PHEM buffer 5 min at room temperature (RT)
3. Carefully wash cells (in chambers if using chamber slides) 2x with PHEM
4. Carefully fix cells (in chambers if using chamber slides) with -20°C MeOH 10 min at RT
5. Remove chambers
6. Wash 4x with 1X PBS shaking for 5 min at RT
7. Incubate with 200 µl Blocking solution (5% NGS, 1% BSA in 1X PBS) 30 min at 37°C in moist chamber
8. Rinse briefly in 1X PBS and add 120 µl (mouse anti-gamma tubulin diluted 1:1000 in 1% NGS, 1% BSA, 1X PBS) 45 min at 37°C in moist chamber
9. Wash 3x 1X PBS 5 min at RT
10. Add 120 µl (sheep anti-mouse-FITC diluted 1:200 in 1% NGS, 1% BSA, 1X PBS) 45 min at 37°C in moist chamber
11. Wash 3x 1X PBS 5 min at RT
12. Counterstain DNA with DAPI [80 ng/ml in 2X SSC] 1min at 37°C
13. Wash 10 min 1X PBS shaking
14. Mount with antifade and cover slip

Notes

1. Steps 1 - 3 can be replaced in some instances by one wash with 1X PBS followed by one wash with PHEM. This is important when doing α -tubulin staining because α -tubulin coagulates into artifactual thick fibers upon permeabilization.
2. This protocol is written for adherent cells grown in chamber slides, but can also be used for cells dropped onto slides (do NOT use hypotonic treated cells if proteins of interest are in the cytoplasm, as they will be destroyed upon dropping cells onto slide). Slides should be allowed to dry completely and then fixed directly in -20°C MeOH for 10 min at RT (step 4).