Immunocytochemistry

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

Reagents

1X Phosphate Buffered Saline (PBS)
Bovine Serum Albumin (BSA)
Boerhinger Mannheim, Cat. 100 350
DAPI
Sigma, Cat. D9542
EGTA
Sigma, Cat. E4378
HEPES
ICN Biomedicals, Inc., Cat. 101926
$MgCl_2$
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	
Total	1000	ml	

*pH to 6.9 with approximately 900 µl 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, add 120 µl (mouse anti-gamma tubulin diluted 1:1000 in 1% NGS, 1% BSA, 1X PBS) and incubate 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) and incubate 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] 1-5 min at RT.

13. Wash 5-10 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).