Immunocytochemistry - Centrosomal Staining

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

Reagents

1X Phosphate Buffered Saline (PBS)
GIBCO – Life Technologies, Cat. 10010-023
Bovine Serum Albumin Fraction V, protease free (BSA)
Roche Diagnostics, Cat. 03 117 332 001
4′,6-Diamidino-2-phenylindole dihydrochloride (DAPI)
Sigma-Aldrich, Cat. D9542
Ethylene glycol-bis(2-aminoethylether)-*N,N,N′,N′*-tetraacetic acid (EGTA)
Sigma-Aldrich, Cat. E4378
1M HEPES Buffer
Sigma-Aldrich, Cat. H3537
2M Magnesium Chloride ($MgCl_2$)
Quality Biological, Inc., Cat. 340-034-060
Methyl Alcohol (MeOH), Anhydrous (Absolute)
MACRON Fine Chemicals, Cat. 3016-02
Monoclonal Anti-Gamma Tubulin (Mouse)
Sigma-Aldrich, Cat. T 6557
Normal Goat Serum (NGS), Sterile
Sigma-Aldrich, Cat. G6767
PIPES BUFFER (**PIPES**) No. P6757
Sigma-Aldrich, Cat. P8658
Sheep Anti-Mouse IgG (whole molecule) FITC
Sigma-Aldrich, Cat. F3008
Triton X-100
Sigma-Aldrich, Cat. T8532
dH_2O, 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]
dH_2O, sterile	978	ml	
Total	1000	ml	

*pH to 6.9 with approximately 900 µl 10M NaOH

0.5 M EGTA

EGTA	3.804	g
Water, sterile	20	ml

*pH to 7.5 to get EGTA into solution

Procedure

1. Wash cells 2x with 1X PBS.
2. Carefully permeabilize cells with 0.5% Triton X-100/PHEM buffer 5 min at room temperature (RT).
3. Carefully wash cells 2x with PHEM.
4. Carefully fix cells with ice-cold (-20˚C) MeOH 10 min at RT.
5. Remove sides, if using chamber slides.
6. Wash 4x with 1X PBS shaking for 5 min at RT (for cells attached to chamber slides, wash in coplin jar).
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 150 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 150 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 (invert the cells grown onto coverslips onto a clean glass slide).

Notes

1. Keep chamber slides intact when cells are grown on them throughout Steps 1- 4. If cells were grown on sterile coverslips, these should remain in tissue culture dishes/wells for Steps 1- 13.

2. 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.

3. This protocol is written for adherent cells grown in chamber slides, but can also be used for fixed cells dropped onto slides (do NOT use hypotonic treated cells if proteins of interest are components of the cytoplasm, as they will be destroyed upon dropping cells onto slide). Slides should be allowed to dry completely and then fixed directly in ice-cold MeOH (-20°C) for 10 min (step 4).