Technical Bulletin

Actin / Tubulin Staining Kit

Catalogue number MAK574

Product Description

The Actin/Tubulin fluorescence staining kit is designed for comprehensive visualization of cellular structures and components. It includes Phalloidin-FITC for precise and specific labeling of actin filaments, supplying valuable insights into cytoskeletal organization. The Anti-α-Tubulin antibody Mouse monoclonal targets α-tubulin, enabling the visualization of microtubule network [1-14]. Complementing this, the Cy3-conjugated Anti-Mouse antibody (not provided) allows for robust detection of specific mouse primary antibodies [15-24]. Additionally, the inclusion of DAPI offers reliable nuclear counterstaining, facilitating precise identification of cellular nuclei across diverse cell types [25-30]. The kit supplies an essential toolkit for fluorescent microscopy applications, yielding detailed and high-quality imaging results for a broad range of research needs.

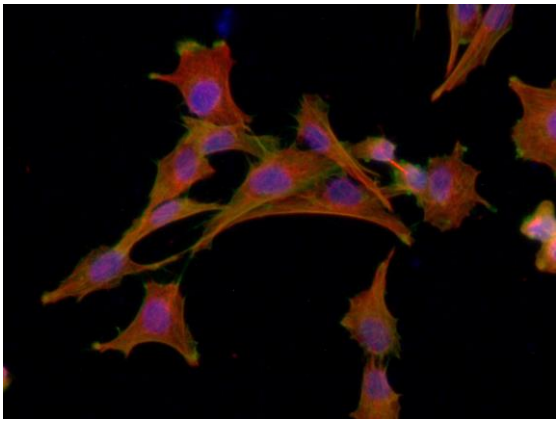


Figure 1: Immunofluorescence of DF-1 Cells. Staining of DF-1 paraformaldehyde Triton™ X-100 fixed cells, merged image. Actin (green) is stained by Phalloidin-FITC (1:100), cell nuclei (blue) are stained by DAPI (1:1000), and tubulin (orange) is stained by an anti-mouse antibody conjugated to Cy3 (1:500).

Components

The kit is sufficient for 200 tests in 96-well plates or 100 tests of cultured adherent cells on coverslips.

- PBS 10X 25 mL
  Catalogue Number MAK574A
- Phalloidin-FITC 0.2 mL
  Catalogue Number MAK574B
- Anti-α-Tubulin antibody, Mouse monoclonal 20 µL
  Catalogue Number MAK574C
- DAPI 20 µL
  Catalogue Number MAK574D

Reagents and Equipment Required but Not Provided

- Cells of DF-1 / Saos-2 / 3T3 (or equivalent) at 70-80% confluency
- Paraformaldehyde
- Triton™ X-100
- Sterile 96 well black well /clear bottom plate
- Microscope glass slides
- Fluorescence microscope, equipped with filters for:
  - DAPI-DNA-complex (Ex/Em=365/450nm)
  - Phalloidin-FITC (Ex/Em=500/520nm)
  - Cy3 (Ex/Em=554/566nm)
- Light protective chamber
- Parafilm
- Anti-Mouse IgG Conjugated to Cy3
- Bovine Serum Albumin
- Fluoromount™ Aqueous Mounting Medium

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the safety data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The product is shipped on dry ice. Store at -20° C upon receipt, protected from light.

Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. Avoid repeated freeze/thaw cycles.

PBS 10X: Dilute 1:10 with ultrapure water. Allow the buffer to reach room temperature before use. Store at 2–8°C. Do not store the diluted buffer; make it fresh for every use.

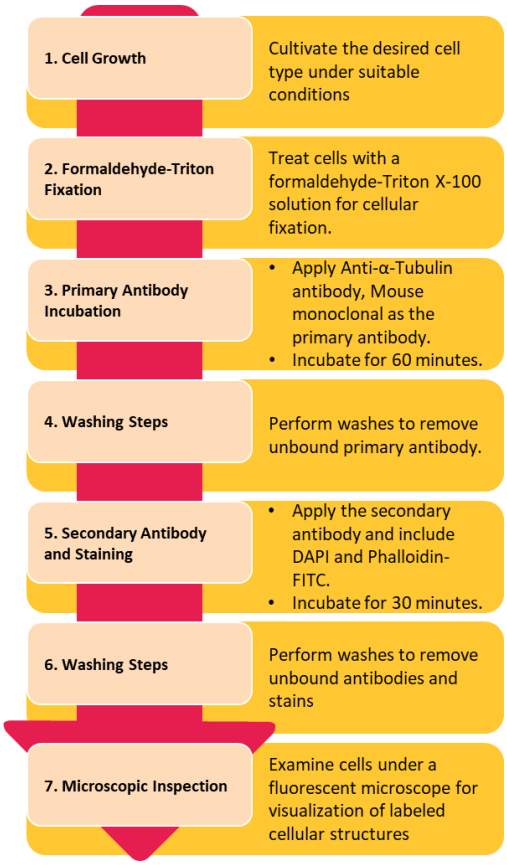
Phalloidin-FITC: Stock concentration of 100X. Allow to reach room temperature before use. Store at -20°C. Do not store the diluted Phalloidin-FITC; make it fresh for every use. Protected from light.

Anti-α-Tubulin antibody, Mouse monoclonal: Stock concentration of 1000X. After the first thawing, divide into aliquots to avoid refreezing. Store at -20°C for long-term storage and at 2-8°C for up to one month.

DAPI: Stock concentration of 1000X. After the first thawing, divide into aliquots

Procedure

Protocol Outline: Visualization of Cellular Structures.



Cell Culturing and Preparation

1. Cultivate cells in suitable media until they reach 70-80% confluency.

2. Fix the cultured cells with 3-4% paraformaldehyde in 1X PBS for 10-20 minutes at room temperature.

3. Briefly wash the cells with 1X PBS.

4. Permeabilize the cells with 0.5% Triton X-100 in 1X PBS for 2-10 minutes at room temperature.

5. Wash the cells three times with 1X PBS, for at least 5 minutes each time.

Primary Antibody Incubation

6. Incubate the cells with a solution of 5% BSA in 1X PBS and 1:1000-2000 Anti-α-Tubulin antibody for 60 minutes at room temperature.

Note: Use a solution containing 0.5 g BSA and 10 µL Anti-α-Tubulin antibody in 10 mL 1X PBS, suitable for either 50 tests of cultured adherent cells on coverslips or 100 tests of cultured adherent cells in a 96-well plate.

Note: Prepare the solution fresh before use.

7. Wash the cells three times with 1X PBS, for at least 5 minutes each time.

Secondary Antibody and Staining

8. Incubate the cells with a solution of 1:50-100 Phalloidin-FITC, 1:1000 DAPI, and 1:500-1000 Cy3-conjugated anti-Mouse antibody for 30 minutes at room temperature, protected from light.

Note: Use a solution containing 100 µL Phalloidin-FITC, 10 µL DAPI, and 20 µL Cy3-conjugated anti-Mouse antibody (1 mg/mL) in 10 mL 1X PBS, suitable for either 50 tests of cultured adherent cells on coverslips or 100 tests of cultured adherent cells in a 96-well plate.

Note: Prepare the solution fresh before use.

Microscopic Inspection

9. If using coverslips, mount them with mounting medium and invert onto glass slides. Wait until it dries.

10. Inspect the cells under a fluorescent microscope using these filters:

    DAPI (Ex/m=365/450nm)

    FITC (Ex/m=500/520nm)

        Cy3 (Ex/m=554/566nm)

11. Perform a control test without any dye or antibody to evaluate the autofluorescence of the sample.

12. Conduct an additional test without the primary antibody to assess the possibility of nonspecific binding of the secondary antibody.

Measurement

1. Incubate the reaction mixture at 37 °C for 30 - 60 minutes.
2. Monitor the absorbance increase with an absorbance plate reader with path check on at OD of 405 nm.

Results

Examples of cell staining:

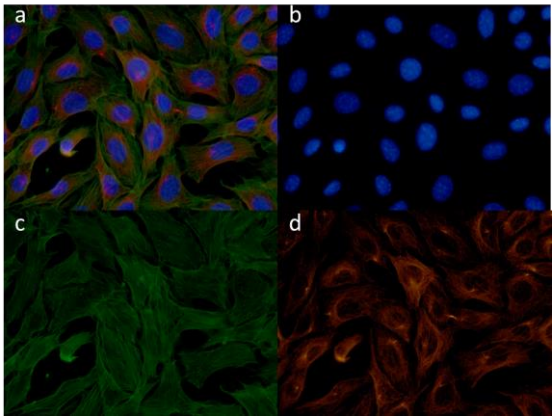


Figure 2: Immunofluorescence of T3T Cells. (a) Staining of T3T cells, merged image. Actin (green) is stained by Phalloidin-FITC, cell nuclei (blue) are stained by DAPI, and tubulin (orange) is stained by an antibody conjugated to Cy3. (b) Staining of T3T cell nuclei by DAPI (blue). (c) Visualization of actin (green) staining using Phalloidin-FITC. (d) Staining of tubulin (orange) by an antibody conjugated to Cy3.

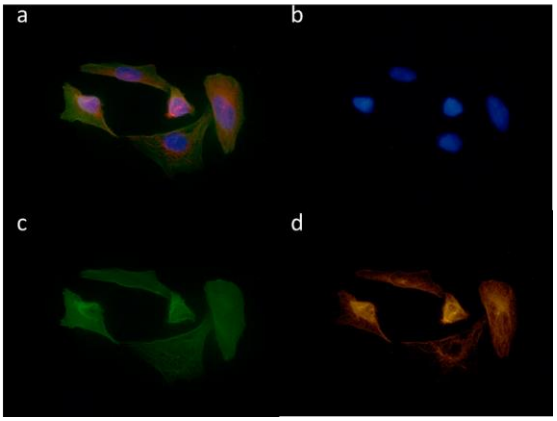


Figure 3: Immunofluorescence of Saos-2 Cells. (a) Staining of Saos-2 cells, merged image. Actin (green) is stained by Phalloidin-FITC, cell nuclei (blue) are stained by DAPI, and tubulin (orange) is stained by an antibody conjugated to Cy3. (b) Staining of T3T cell nuclei by DAPI (blue). (c) Visualization of actin (green) staining using Phalloidin-FITC. (d) Staining of tubulin (orange) by an antibody conjugated to Cy3.

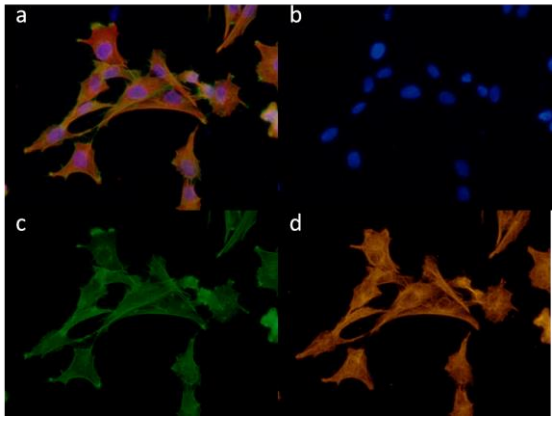


Figure 4: Immunofluorescence of DF-1 Cells. (a) Staining of DF-1 cells, merged image. Actin (green) is stained by Phalloidin-FITC, cell nuclei (blue) are stained by DAPI, and tubulin (orange) is stained by an antibody conjugated to Cy3. (b) Staining of T3T cell nuclei by DAPI (blue). (c)Visualization of actin (green) staining using Phalloidin-FITC. (d) Staining of tubulin (orange) by an antibody conjugated to Cy3

References

1. 1 Faulstich, H et al. *Journal of muscle research and cell motility* vol. 9,5 (1988): 370-83.
2. ABUL-HAJ, S K et al. *The New England journal of medicine* vol. 269 (1963): 223-7.
3. Heidecker, M et al. *Biochemistry* vol. 34,35 (1995): 11017-25.
4. Wulf, E et al. *Proceedings of the National Academy of Sciences of the United States of America* vol. 76,9 (1979): 4498-502.
5. Small, J V et al. *Journal of cell science* vol. 89 (Pt 1) (1988): 21-4.
6. Faulstich, H et al. *Experimental cell research* vol. 144,1 (1983): 73-82.
7. Lawson, M A, and F R Maxfield*Nature* vol. 377,6544 (1995): 75-9.
8. Honda, K et al. *The Journal of cell biology* vol. 140,6 (1998): 1383-93.
9. Carulli, Giovanni et al. *American journal of hematology* vol. 81,5 (2006): 318-23.
10. Kopitar, Andreja N et al. *Frontiers in immunology* vol. 10 1632. (2019).
11. Horobin, Adele J et al. *The Journal of investigative dermatology* vol. 126,6 (2006): 1410-8.
12. Qiao, Fei et al. *Journal of experimental botany* vol. 61,14 (2010): 4021-31.
13. Tan, Kun et al. *PloS one* vol. 10,6 e0130382, (2015).
14. Waggoner, A et al. *Methods in cell biology* vol. 30 (1989): 449-78.
15. Blose, S H et al. *The Journal of cell biology* vol. 98,3 (1984): 847-58.
16. Breitling, F, and M Little. *Journal of molecular biology* vol. 189,2 (1986): 367-70.
17. Wolff, A et al. *Biology of the cell* vol. 63,3 (1988): 319-26.
18. Serrano, L et al. *Analytical biochemistry* vol. 159,2 (1986): 253-9.
19. LeDizet, M, and G Piperno. *The Journal of cell biology* vol. 103,1 (1986): 13-22.
20. Dutcher, S K. *Current opinion in cell biology* vol. 13,1 (2001): 49-54.
21. McKean, P G et al. *Journal of cell science* vol. 114,Pt 15 (2001): 2723-33.
22. Eiserich, J P et al. *Proceedings of the National Academy of Sciences of the United States of America* vol. 96,11 (1999): 6365-70.
23. Perez, R G et al. *The Journal of neuroscience : the official journal of the Society for Neuroscience* vol. 17,24 (1997): 9407-14.
24. Machaca, K, and S Haun. *The Journal of biological chemistry* vol. 275,49 (2000): 38710-5.
25. Yu, Ting-Ting et al. *Cell & bioscience* vol. 11,1 47. (2021)
26. Jones, Mark et al. *Cell transplantation* vol. 29 (2020): 963689720923578.
27. Zeng, Wen et al. *Frontiers in cellular neuroscience* vol. 15 638351. (2021).
28. Kampanis, Vasileios et al. *Cells*. 10,1 32. (2020).
29. Zhang, June et al. *Chemosphere* vol. 256 (2020): 127038.
30. Nesterenko, Maksim A et al. *Parasites & vectors* vol. 13,1 559. (2020).

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
1. No staining	Inadequate or expired reagents, insufficient antibody or dye incubation time, or improper sample preparation.	Verify reagent expiration dates, ensure appropriate reagent storage, optimize antibody and dye concentration, and review sample preparation protocols.
2. Weak staining	Low antibody or dye concentration, or improper washing steps.	Optimize antibody and dye concentration, extend antibody-antigen or dye incubation time, and ensure thorough and appropriate washing steps.
3. Staining too strong	Excessive antibody or dye concentration, prolonged incubation, or high non-specific binding.	Optimize antibody and dye dilution, reduce incubation time, and validate blocking procedures to minimize non-specific binding.
4. Uneven Staining	Unequal distribution of reagents, inconsistent washing, or uneven sample fixation.	Ensure consistent reagent distribution, optimize washing steps, and review sample fixation techniques for uniformity.
5. Non-specific Binding	Inadequate blocking, cross-reactivity of 2° antibody.	Enhance blocking procedures, validate 2° antibody, and optimize conditions to reduce cross-reactivity.
6. High Autofluorescence	Inadequate sample fixation, sample impurities, or autofluorescent contaminants.	Optimize sample fixation protocols, minimize sample impurities, and use appropriate controls to identify autofluorescent contaminants.
7. Excessive background staining	Non-specific binding of antibodies or dyes, contamination, or improper blocking.	Enhance blocking steps, review sample preparation for contamination, and ensure proper 2° antibody specificity.

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