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Actin Cytoskeleton Staining Protocol

Assay Description

The actin cytoskeleton is a dynamic network of actin filaments that provides structural support, shape, and motility to eukaryotic cells. Composed of polymerized actin protein, it plays a critical role in various cellular processes, including cell division, intracellular transport, and signal transduction. The actin cytoskeleton is essential for maintaining cell integrity, enabling cell movement, and facilitating interactions with the external environment.

Staining the actin cytoskeleton with a fluorescence probe can facilitate the visualization of cell morphology using a fluorescence microscope in both 2D and 3D culture. Here we present a protocol to stain the actin cytoskeleton in cells cultured in 3D hydrogels for fluorescence imaging.

Materials Needed

- PureCol® (Cat #5005)
- Cells/cell lines of interest
- Cell culture media
- 4% paraformaldehyde (ThermoFisher Cat # 047392-9M)
- Bovine Serum Albumin (BSA, Sigma-aldrich Cat #A9418)
- Alexa Fluor™ 546 Phalloidin (ThermoFisher Cat # A22283)
- Triton X-100 (ThermoFisher Cat # A16046-AE)
- DAPI (ThermoFisher Cat # 62248)
- 1× PBS
- Cell culture plates
- Centrifuge tubes
- Serological pipettes
- Micropipette and tips
- Orbital shaker
- Fluorescence microscope

Procedure

1. Culture cells in PureCol® or other collagen hydrogel following the Hydrogel Cell Culture Protocol.
2. Remove cell culture medium from the hydrogel wells and rinse the hydrogels with 1X PBS twice to wash off the residual culture medium.
3. Fixation: add 4% formaldehyde solution to the hydrogel wells and incubate the samples at room temperature for 1 h.
4. Remove the formaldehyde solution and wash the hydrogels with 1X PBS three times. After washing, the samples can be stored in 1X PBS at 4 °C before staining.
5. Prepare the blocking buffer (5 w/v% BSA solution in 1X PBS) and the permeabilization buffer (0.1 v/v% Triton-X diluted in 1X PBS).
6. Remove the 1X PBS and add 0.1 v/v% Triton-X to the samples and incubate for 10 minutes at room temperature to permeabilize the cell membrane.
7. After permeabilization, wash the samples three times with 1X PBS.
8. Add the BSA blocking buffer to the samples and incubate for 30 minutes at room temperature.
9. After blocking, stain the samples with Alexa Fluor 546-phalloidin (1:50 diluted with blocking buffer) and leave them covered in the dark for 30 minutes.

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10. Prepare the washing buffer (1 w/v% BSA) and wash the stained samples twice, followed by a 1X PBS wash once.

11. Stain samples with DAPI (1:950 with blocking buffer) for 5 minutes at room temperature on an orbital shaker.

12. Wash the samples with 1X PBS three times and proceed to imaging.

13. Image the stained samples with a fluorescence microscope using the RFP channel (Ex: 531 nm, Em: 593 nm) for actin cytoskeleton and DAPI channel (Ex: 377 nm, Em: 447 nm) for cell nucleus.

14. After imaging, cover the plate with aluminum foil and store the stained samples at 4°C.

Notes:

1. It is recommended to prepare the permeabilization, blocking, and washing buffer as stocks prior to the experiment. Stock solutions can be stored at 4°C for up to 2 weeks.

2. Solubilized BSA can be centrifuged at 500 × g for 5 min to separate the microbubbles and precipitate the undissolved impurities in the solution. The solution can later be filtered through a 0.2 µm filter for further purification before use.

Sample images

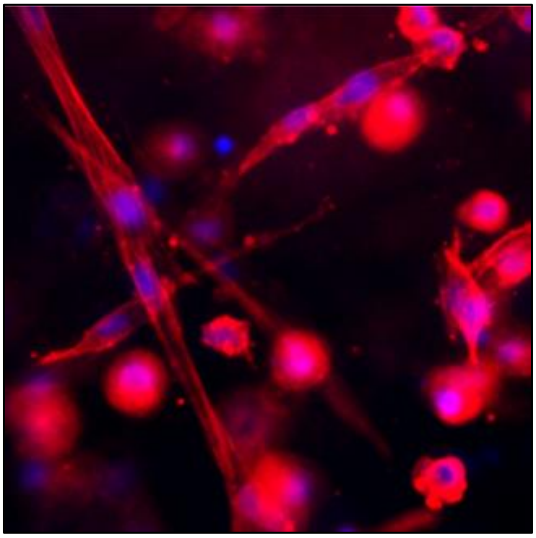

Fig.1. MDA-MB-231 breast cancer cells cultured in PureCol® hydrogel.

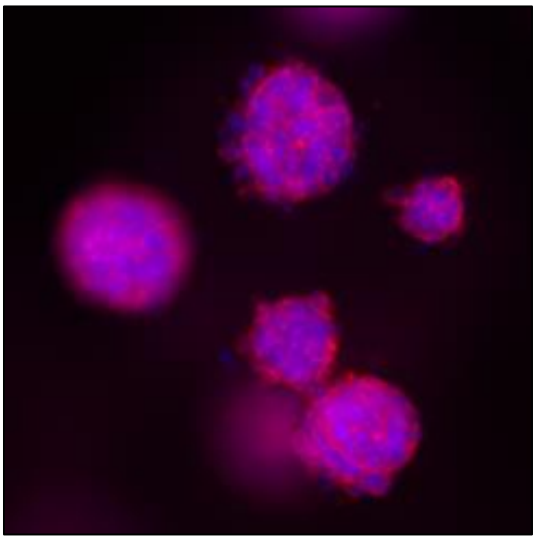

Fig.2. MCF-7 breast cancer cells cultured in PureCol® hydrogel.

