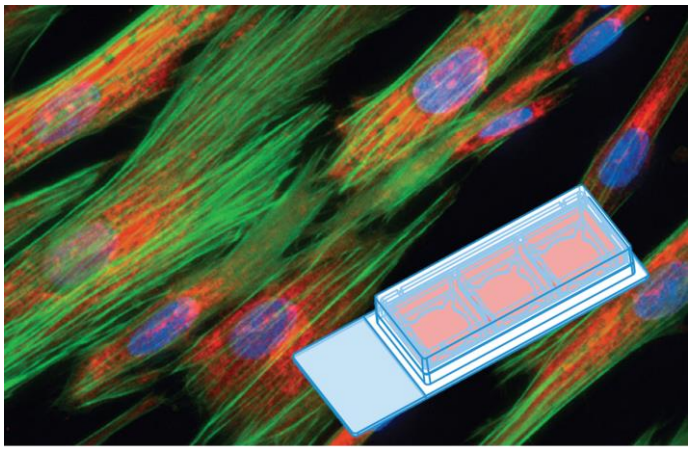
Immunofluorescence Staining Protocol for 3 Well Chamber, removable

This Application Note presents a simple protocol for the cultivation, fixation, and staining of cells using the 3 Well Chamber, removable. MCF-7 cells were cultivated and fixed with a formalin solution. Cell nuclei were stained with DAPI and actin skeletons with DYE-490 phalloidin. The amount of staining solution needed can be reduced by using the round 15 mm coverslip. It is possible to probe for other intracellular structures by immunocytochemistry using primary and secondary antibody staining.

1. Material

The material and reagents used are listed below. Additionally, an inverted or upright fluorescence microscope equipped with the appropriate filter set is needed.

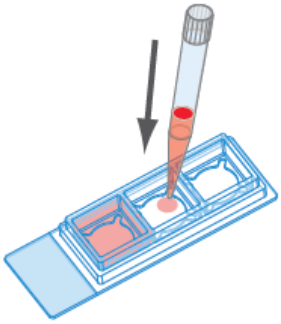
- 3 Well Chamber, removable (ibidi, 80381)
- Coverslips and Coverslip Pick-Up Tool for 3 Well Chamber, removable, Ø 15 mm (ibidi, 10815)
- Coverslips for Chambers, removable, 24 mm x 60 mm (ibidi, 10811)
- Cells: MCF-7 (CLS, 300273)
- Cell culture medium
- Cell culture reagents
- PBS
- Formalin solution neutral buffered, 10 % (Sigma, HT5011)
- Staining reagents
  - DAPI (Sigma, D9542)
  - DYE-490 Phalloidin (Dynomics, 490-33)
- Mounting medium: Fluoroshield™ (Sigma, F6182)



2. Experimental Protocol

Step 1:

The correct seeding concentration for your cell line has to be defined in pre-experiments. Depending on your cell type, application of 2-6x10^4 cells/ml should result in a confluent monolayer within 2-3 days.

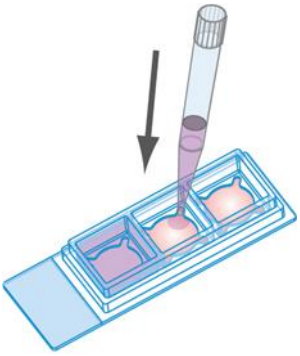


Seed cells and incubate

1. Unpack the 3 Well Chamber, removable under sterile conditions.
2. Trypsinize and count cells as usual. A cell concentration of 3x10^4 cells/ml was used for MCF-7.
3. Apply 1100 µl of the cell suspension into each well of the slide. Avoid shaking as this will result in an inhomogeneous distribution of cells. For a more homogenous cell distribution use the round 15 mm coverslip as described in the Instruction 3 Well Chamber, removable.
4. Cover the slide with the supplied lid. Incubate at 37°C and 5% CO_2.
5. Cultivate your cells for at least 24 hours, or until a confluent monolayer is established.

Step 2:

Fixation is the first step of a staining procedure. The goal is to maintain cells, cellular formations, or tissue in their current state and to preserve the preparation by chemical reagents over an extended period.



Fixation

1. Carefully aspirate the cell culture medium.
2. Wash twice with PBS.
3. Add 1100 µl formalin solution.
4. Incubate for 30 minutes at room temperature.
5. Carefully aspirate the formalin solution.
6. Wash three times with PBS.

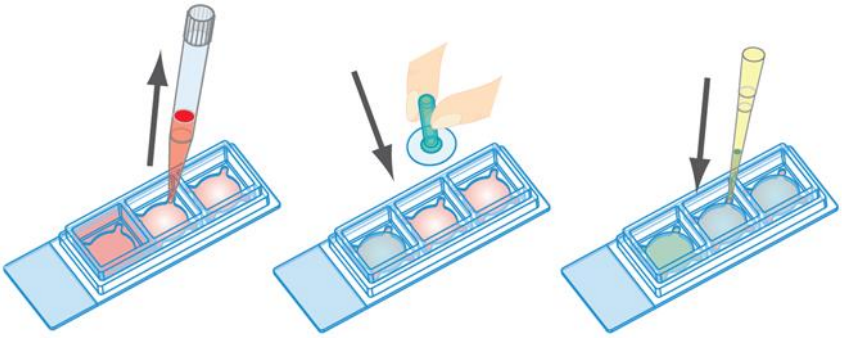
Step 3:

The staining reagent(s) should to be chosen based on the cell structure(s) of interest. DAPI and DYE-490 phalloidin were used in this protocol to stain nuclei and the actin skeletons of MCF-7 cells.

1. Prepare your staining solution:
    - PBS
    - DYE-490 Phalloidin (one Unit per Slide ≙ 1 Unit/450 µl)
    - DAPI 1 µg/ml
2. Carefully aspirate the PBS.
3. Grab the round 15 mm coverslip with the Coverslip Pick-Up Tool and place it

onto the rounded edge inside the chamber well.

4. Pipette 150 µl of the staining solution into the well by placing the pipette tip into one of the corner notches.
5. Incubate for 30 minutes at room temperature in the dark.

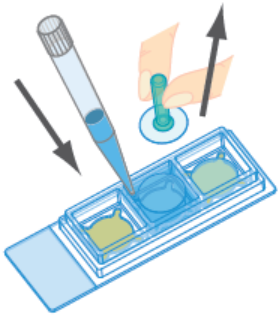


Remove medium *Place Glass Coverslip* *Staining*

Step 4:

Washing your samples after staining minimizes the background signal.

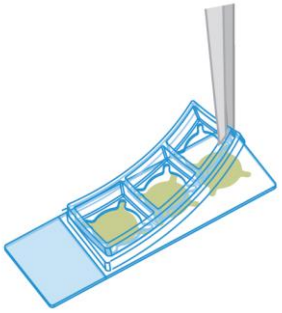
1. Removing the coverslip by adding 950 µl of buffer in one of the corner notches.
2. Grab the floating coverslip with the Coverslip Pick-Up Tool or tweezers and remove it from the well.
3. If necessary, repeat the washing step by aspirating the buffer and pipetting 1100 µl of buffer in each well. The round 15 mm coverslip is not needed for additional washing steps.



Wash and remove floating glass coverslip

Step 5:

Starting from one edge, carefully remove the silicone gasket by hand or with tweezers. This step should be performed in a slow, steady movement to avoid damaging the cell layer.



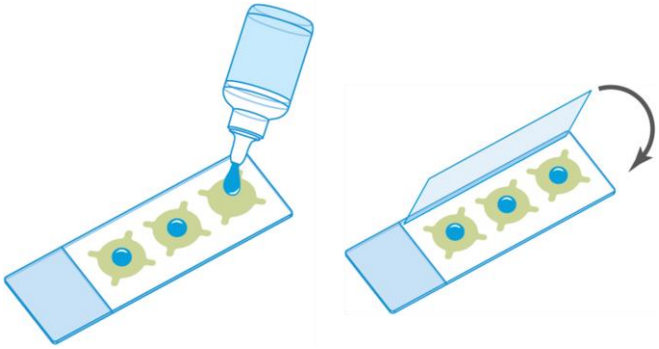
Remove the chamber

Step 6:

After completing the staining procedure, the samples must be mounted prior to imaging with a microscope. This also prevents its dehydration.

1. Remove any excess medium from the sample by tapping the side of the slide on to a clean laboratory wipe.

2. Apply the mounting medium to your sample. Cover the mounted sample with a 24 mm x 60 mm coverslip by carefully lowering the coverslip onto the mounting medium to avoid trapping any air bubbles. A hardening mounting medium such as Fluoroshield™ (Sigma-Aldrich), Vectashield® (Vector Laboratories Inc.), or ProLong Antifade® (ThermoFisher Scientific) is recommended.
3. Allow the mounting medium to cure.



3. Microscopy

MCF-7 cells were cultivated using the 3 Well Chamber, removable. Cellular structures were stained with DAPI and DYE 490 phalloidin. The round 15 mm coverslip reduced the amount of staining solution needed. Mounting the slides with a hardening mounting medium preserved the samples for long term storage.

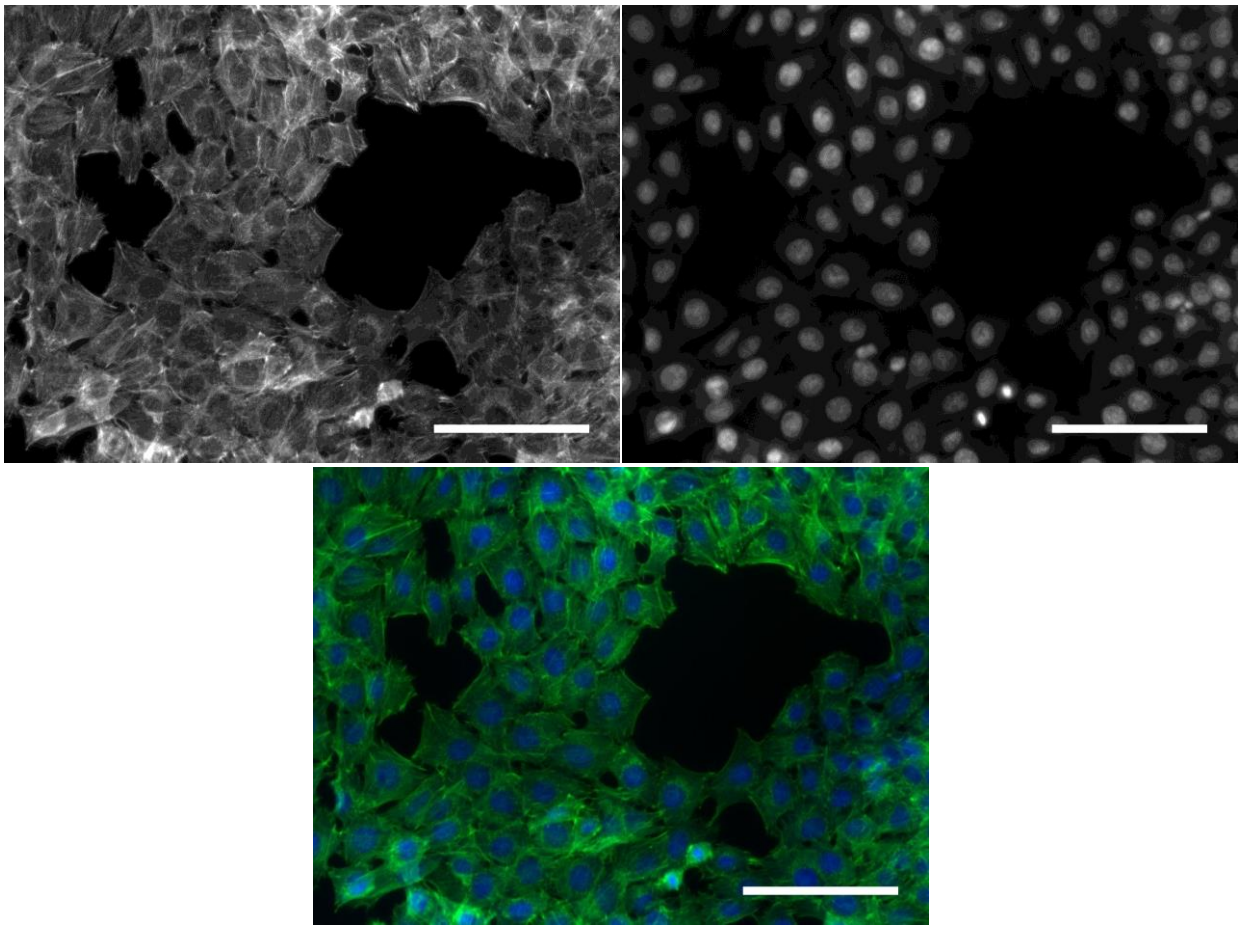


Figure 1 The actin skeletons of MCF-7 cells were stained with DYE 490 phalloidin (upper left) and the nuclei were stained with DAPI (upper right). The lower picture shows the composite image, with the nuclei in blue and the actin skeletons in green. (Scale bar: 100 µm)