

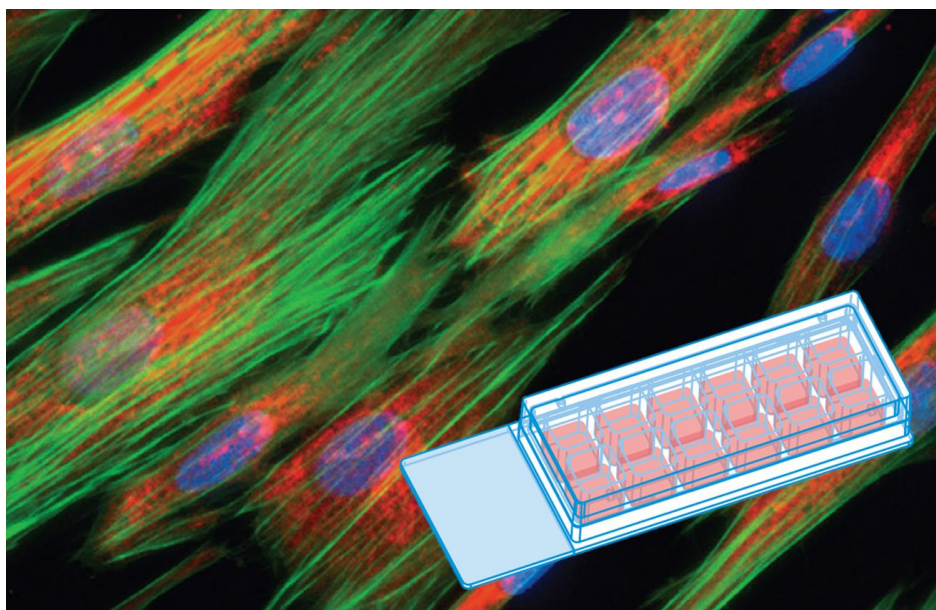
## Immunofluorescence Staining Protocol for 12 Well Chamber, removable

This Application Note presents a simple protocol for the cultivation, fixation, and staining of cells using the 12 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. 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.

- 12 Well Chamber, removable (ibidi, 81201)
- 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 (Dynamics, 490-33)
- Mounting medium: Fluoroshield™ (Sigma, F6182)

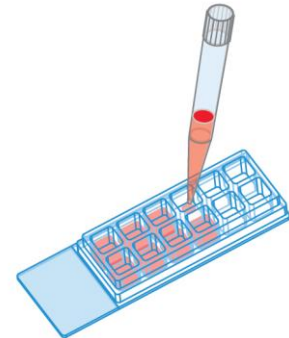


## 2. Experimental Protocol

### Step 1:

The correct seeding concentration for your cell line has to be defined in pre-experiments. Depending on your cell type, application of  $2-6 \times 10^4$  cells/ml should result in a confluent monolayer within 2-3 days.

1. Unpack the 12 Well Chamber, removable under sterile conditions.
2. Trypsinize and count cells as usual. A cell concentration of  $5 \times 10^4$  cells/ml was used for MCF-7.
3. Apply 250  $\mu$ l of the cell suspension into each well of the chamber. Avoid shaking as this will result in an inhomogeneous distribution of cells.
4. Cover the slide with the supplied lid. Incubate at 37°C and 5% CO<sub>2</sub>.
5. Cultivate your cells for at least 24 hours, or until a confluent monolayer is established.

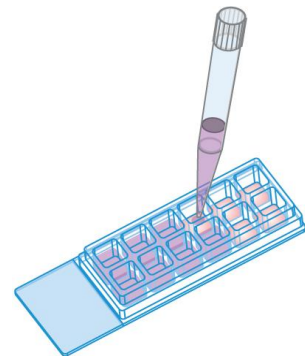


*Seed cells and incubate*

### 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.

1. Carefully aspirate the cell culture medium.
2. Wash twice with PBS.
3. Add 250  $\mu$ l formalin solution.
4. Incubate for 30 minutes at room temperature.
5. Carefully aspirate the formalin solution.
6. Wash three times with PBS.

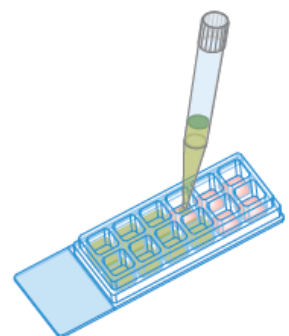


*Fixation*

### Step 3:

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

1. Prepare your staining solution:
  - PBS
  - DYE-490 Phalloidin (one Unit per Slide  $\cong$  1 Unit/3ml)
  - DAPI 1  $\mu$ g/ml
2. Carefully aspirate the PBS.
3. Pipette 250  $\mu$ l of the staining solution into each well



*Staining*

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of the chamber.

4. Incubate for 30 minutes at room temperature in the dark.

### Step 4:

Washing your samples after staining minimizes the background signal.

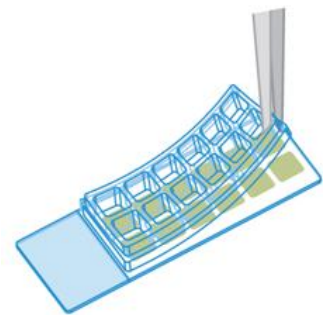
1. Carefully aspirate the staining solution.
2. Add 250  $\mu$ l buffer.
3. Carefully aspirate the buffer.
4. Repeat steps 2 and 3 at least once.



*Wash*

### 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.

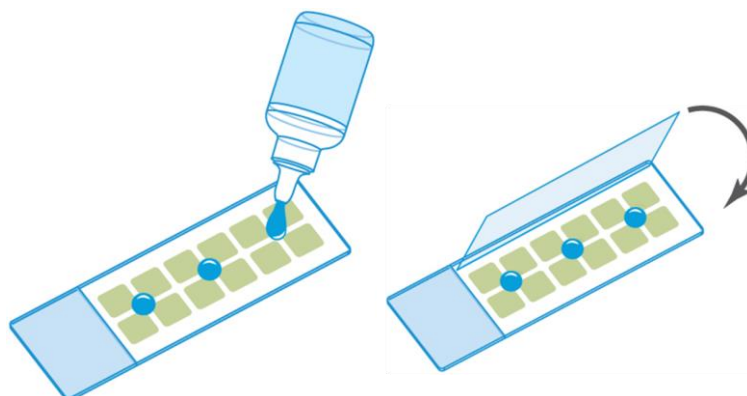


*Remove the chamber*

### Step 6:

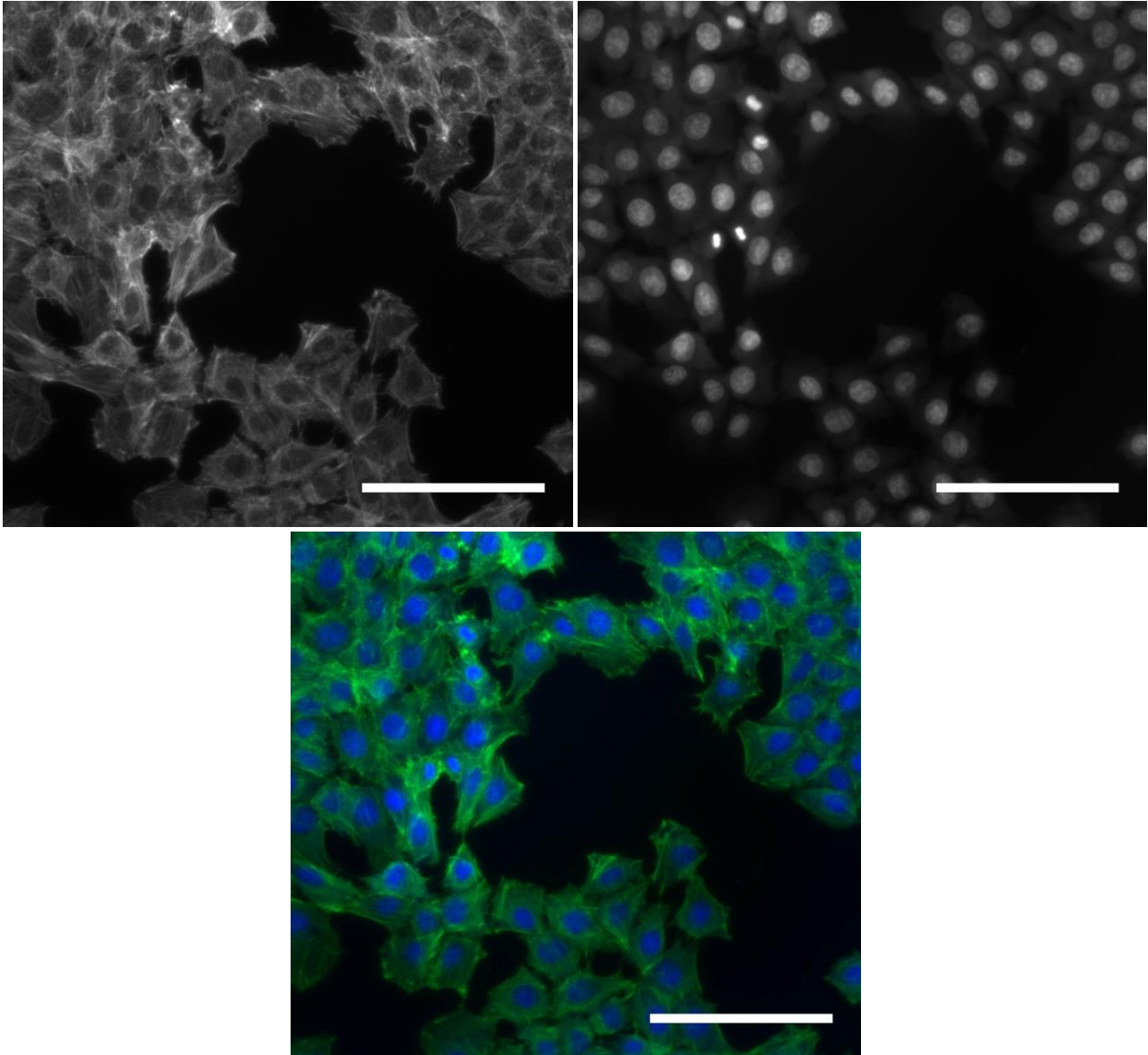
After completing the staining procedure, the samples must be mounted prior to imaging with a microscope. This step also prevents dehydration of the sample.

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 12 Well Chamber, removable. Cellular structures were stained with DAPI and DYE 490 phalloidin. 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  $\mu$ m)