

Immunofluorescence Microscopy Protocol Version 2

BioLegend, Inc.

Abstract

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Guidelines

Materials and Buffers

1. Chamber slides or cover slips

2. Fixation solution: 4% Paraformaldehyde, in PBS

3. Permeablization solution: 0.5% Triton X-100 in PBS

4. Antibody dilution solution: 5%FBS in PBS

Protocol

Sample Preparation

Step 1.

Grow cultured cells on coverslips or chamber slides overnight, or add appropriate amount of cells to poly-L-lysine coated chamber slides and incubate at least 30 min at 37°C, at the time of fixation cells should be 50% confluent.

O DURATION

00:30:00

Sample Preparation

Step 2.

Rinse cells briefly in PBS.

Sample Preparation

Step 3.

Fix cells by incubation with 4% Paraformaldehyde, in PBS for 15 min at room temperature.

O DURATION

00:15:00

Sample Preparation

Step 4.

Rinse in PBS for 5 min (1/3).

O DURATION

00:05:00

Sample Preparation

Step 5.

Rinse in PBS for 5 min (2/3).

O DURATION

00:05:00

Sample Preparation

Step 6.

Rinse in PBS for 5 min (3/3).

O DURATION

00:05:00

Sample Preparation

Step 7.

Add 0.5% Triton X-100 in PBS and incubate at room temperature for three to five min.

O DURATION

00:03:00

Sample Preparation

Step 8.

Rinse in PBS for 5 min (1/3).

© DURATION

00:05:00

Sample Preparation

Step 9.

Rinse in PBS for 5 min (2/3).

© DURATION

00:05:00

Sample Preparation

Step 10.

Rinse in PBS for 5 min (3/3).

O DURATION

00:05:00

Sample Blocking

Step 11.

Block samples in 5% FBS in PBS at room temperature for one hour.

O DURATION

01:00:00

Sample Staining

Step 12.

Dilute the primary antibody to the recommended concentration/dilution in 5% FBS/PBS.

Sample Staining

Step 13.

Add 200 μ l per well (8 wells) to the chamber slides and incubate two to three hours at room temperature or overnight at 4°C.

O DURATION

03:00:00

Sample Staining

Step 14.

Rinse in PBS for 5 min (1/3).

O DURATION

00:05:00

NOTES

Kelsey Knight 09 May 2017

NOTE: If using primary antibodies directly conjugated with fluorochrome, then skip to step 22 "Coverslip with anti-fade mounting medium."

Sample Staining

Step 15.

Rinse in PBS for 5 min (2/3).

O DURATION

00:05:00

Sample Staining

Step 16.

Rinse in PBS for 5 min (3/3).

O DURATION

00:05:00

Sample Staining

Step 17.

Prepare fluorochrome-conjugated secondary antibody in 5% FBS/PBS according to the recommended manufacturer specification data sheet, and add 200 µl per well (8 wells) to the chamber slides.

Sample Staining

Step 18.

Incubate the samples for 1 hour, at room temperature, in the dark.

O DURATION

01:00:00

Sample Staining

Step 19.

Rinse in PBS for 5 min (1/3).

O DURATION

00:05:00

Sample Staining

Step 20.

Rinse in PBS for 5 min (2/3).

O DURATION

00:05:00

Sample Staining

Step 21.

Rinse in PBS for 5 min (3/3).

O DURATION

00:05:00

Sample Staining

Step 22.

Coverslip with anti-fade mounting medium.

Sample Staining

Step 23.

Seal slides with nail polish.