

Product Information

Prestige Antibodies®

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ANTIBODIES

Immunofluorescence Procedure

Product Description

A large number of the Prestige Antibodies® have been used in subcellular localization studies by immunofluorescence (IF) staining of three cell lines: A-431, U-2 OS, and U-251MG. Each cell line is stained with the Prestige Antibody, two organelle probes specific for the endoplasmic reticulum and microtubules, as well as counterstained with the nuclear probe DAPI.

Preparation Instructions

1× PBS – 8.1 mM Na₂HPO₄, 1.5 mM KH₂PO₄, 137 mM NaCl, and 2.7 mM KCl, pH 7.2

Cell Seeding – All washes are performed at room temperature (RT).

1. A glass-bottomed, multiwell plate is coated with fibronectin (12.5 µg/ml) for 1 hour at room temperature.
2. Cells are seeded (10,000–15,000 cells per well) and incubated at 37 °C in humidified air with 5.2% CO₂ for 2 hours.

Procedure

Primary Antibodies:

- Prestige Antibody (working concentration of 2 µg/ml)
Note: The dilution of the primary antibody is a guideline only. Optimal dilution must be determined by the user.
- Chicken anti-Calreticulin polyclonal antibody
- Mouse anti-α-Tubulin monoclonal antibody

Secondary Antibodies:

- anti-mouse IgG–Alexa Fluor® 555 produced in goat
- anti-chicken IgG–Alexa Fluor 647 produced in goat
- anti-rabbit IgG–Alexa Fluor 488 produced in goat

Immunostaining

1. Growth medium is removed and the cells are washed in 1× PBS.
2. The cells are fixed for 15 minutes in ice cold, 4% paraformaldehyde, pH 7.2–7.3, in growth medium supplemented with 10 % fetal bovine serum (FBS).
3. The cells are permeabilized 3 times for 5 minutes each, with 0.1% TRITON® X-100 in 1× PBS.
4. The cells are washed with 1× PBS and incubated overnight at 4 °C with the primary antibodies in 1× PBS supplemented with 4% FBS.
5. The following day the cells are washed 4 times for 10 minutes each, with 1× PBS and incubated for 1.5 hours at room temperature with the secondary antibodies in 1× PBS supplemented with 4% FBS. Note: The secondary antibodies are fluorescently labeled and thus light sensitive. The sample should be kept in dim light in this as well as the following steps.
6. The cells are counterstained for 4 minutes with the nuclear stain DAPI (0.3 µM in 1× PBS).
7. The cells are washed 4 times for 10 minutes each, with 1× PBS and then mounted in glycerol with 10% 10× PBS.

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KB,LPG,MAM 06/09-1

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