

Protocol for growing and staining cells for immunofluorescence

Enabling cell growth on the Nunc Lab-Tek II CC² Chamber Slide System

The Thermo Scientific™ Nunc™ Lab-Tek™ II CC² Chamber Slide System allows for growth and staining of cells on a single surface. A chamber slide consists of a removable polystyrene media chamber attached to a glass slide treated for adherent cell culture. The Nunc Lab-Tek II CC² Chamber Slide System features a chemically modified growth surface that mimics the characteristics of polylysine coating. This setup eases the workflow and reduces processing time by eliminating the need for prior coating and drying of glass coverslips before seeding cells. The room temperature–stable chamber slide system is also ideal for live-cell imaging. To meet the needs of researchers, these chamber slides are available in 1-, 2-, 4-, and 8-well formats.

Materials

Nunc Lab-Tek II CC² Chamber Slide System

Number of wells	Culture surface area (per well)	Suggested working volume (per well)	Suggested cell seeding density/well	Cat. No.
1	8.6 cm ²	4 mL	10 x 10 ⁴	154739
2	4.0 cm ²	2 mL	5 x 10 ⁴	154852
4	1.7 cm ²	1 mL	2.5 x 10 ⁴	154917
8	0.7 cm ²	0.5 mL	1 x 10 ⁴	154941

Other materials required:

- Thermo Scientific™ Pierce™ 16% Formaldehyde (Cat. No. 28908)
- Thermo Scientific™ Tween™ 20 Solution (Cat. No. 003005)
- Thermo Scientific™ Triton™ X-100 Surfact-Amps™ Detergent Solution (Cat. No. 28314)
- Thermo Scientific™ Pierce™ Bovine Serum Albumin (BSA) (Cat. No. 23209)
- Invitrogen™ ProLong™ Diamond Antifade Mountant with DAPI (Cat. No. P36962)
- Gibco™ Phosphate-Buffered Saline (PBS, 10X), pH 7.4 (Cat. No. 70011044)

Buffers to be prepared:

- 1X PBS (dilute 10X PBS with double-distilled water)
- Blocking buffer: 1% BSA in 1X PBS
- Wash buffer: 1X PBST (0.05% Tween 20 detergent in 1X PBS)
- Permeabilization solution: 0.1% Triton X-100 detergent in 1X PBS

Staining protocol

1. Discard the cell culture medium by inverting the slide and gently tapping it on a paper towel to remove the remaining medium.
2. Fix the cells with 4% formaldehyde (diluted in 1X PBS—prepare fresh) for 10 min at room temperature (fixation time can be increased to 20 min depending on the cell line). Fixed cells can be stored at 4°C for up to 1 week.
3. Discard the formaldehyde solution, and wash the wells with 1X PBS three times.
4. (Optional) Quench the formaldehyde using 50 mM NH₄Cl for 15 min at room temperature to eliminate any free aldehyde groups that could nonspecifically bind antibody. Wash the wells with 1X PBS three times.
5. Permeabilize the cells using permeabilization solution for 15 min at room temperature (permeabilization time can be increased to 30 min depending on the cell line).
6. Discard the permeabilization solution, and wash the wells with 1X PBS three times.
7. Block the cells using blocking buffer for 1 hour at room temperature.
8. Incubate the cells with the appropriate amount of primary antibody diluted in 0.1% BSA in 1X PBS at room temperature for 3 hours or at 4°C overnight.
9. Discard the primary antibody solution, and wash the cells with wash buffer three times.
10. Incubate the cells with the appropriate amount of secondary antibody diluted in 0.1% BSA in 1X PBS for 1 hour at room temperature in the dark.
11. (Optional) Counterstain with Invitrogen™ rhodamine phalloidin (Cat. No. R415) (1:300 in 0.1% BSA in 1X PBS) for 1 hour at room temperature. This can be done with the secondary antibody incubation.
12. Wash the wells with wash buffer three times, and finally once with 1X PBS.
13. Remove the media chamber carefully with the provided chamber removal tool, and scrape the glue off using a razor blade or a utility knife.
14. Mount the cells using mounting medium and carefully place the required size of coverslip on top, making sure there is no air bubble.
15. Cure the slide overnight at room temperature in the dark, and image the following day.

Note: We recommend using Invitrogen™ NucBlue™ Live ReadyProbes™ Reagent (Cat. No. R37605) as a nuclear stain for live-cell imaging.

Imaging examples

Cell lines were cultured on Nunc Lab-Tek II CC² chamber slides and stained for proteins localized to various cellular compartments using the protocol described. Representative images are shown here.

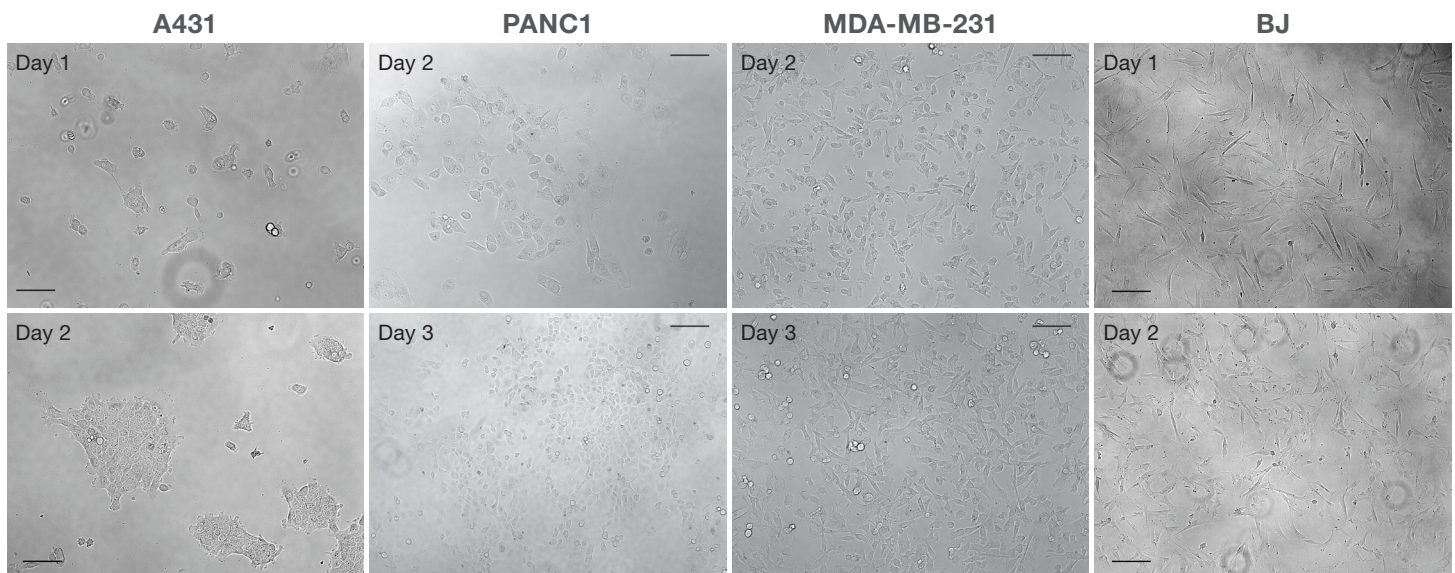


Figure 1. Brightfield images of cell lines. Slow-growing (A431, PANC-1) and fast-growing (MDA-MB-231, BJ) cells were seeded at the density recommended in “Materials”. Brightfield images were captured on the indicated days using an Invitrogen™ EVOS™ XL Imaging System. Scale bar: 500 µm.

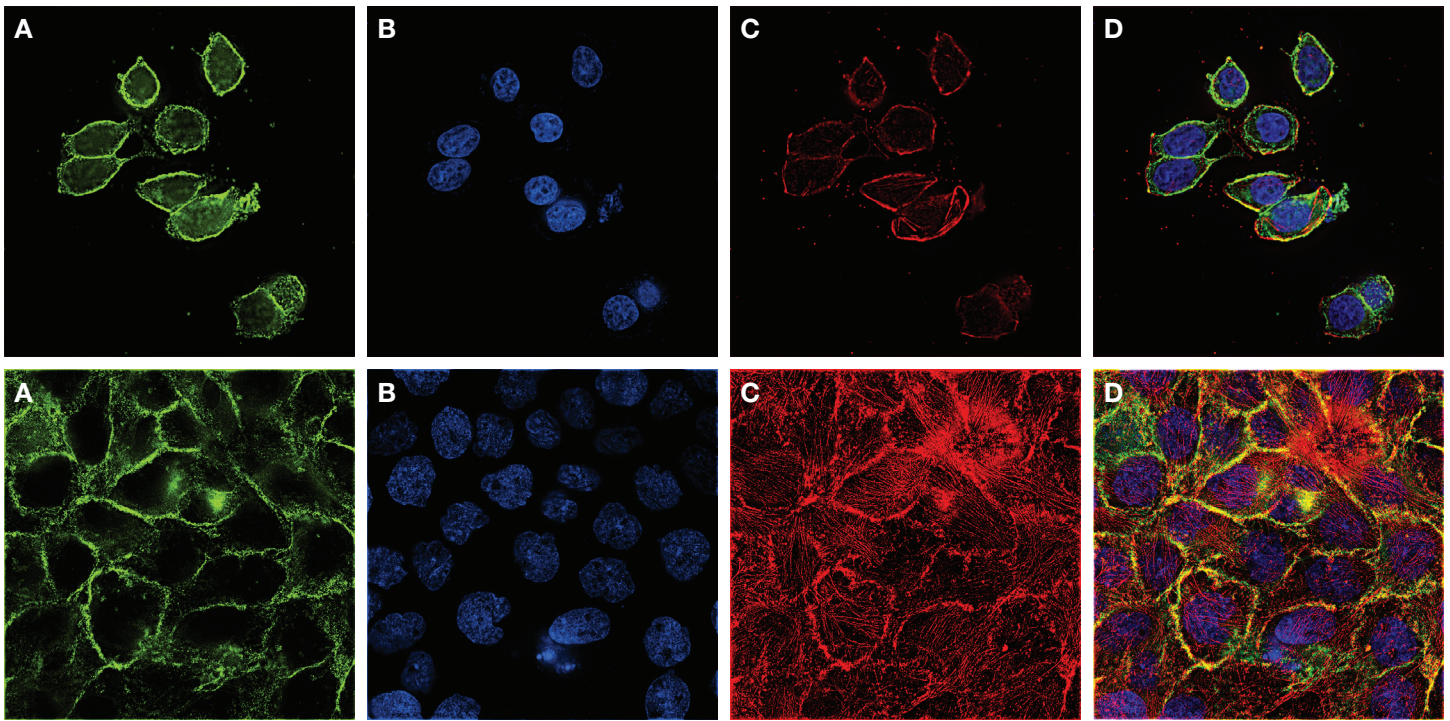


Figure 2. Plasma membrane marker expression. Top row: 90% confluent log-phase HT-29 cells were stained with Invitrogen™ EpCAM Monoclonal Antibody (Cat. No. MA5-12442). Bottom row: 90% confluent log-phase A431 cells were stained with Invitrogen™ EGFR Antibody (Cat. No. AHR5062). (A) Protein of interest, (B) DNA stained with DAPI, (C) F-actin stained with rhodamine phalloidin, (D) merged image.

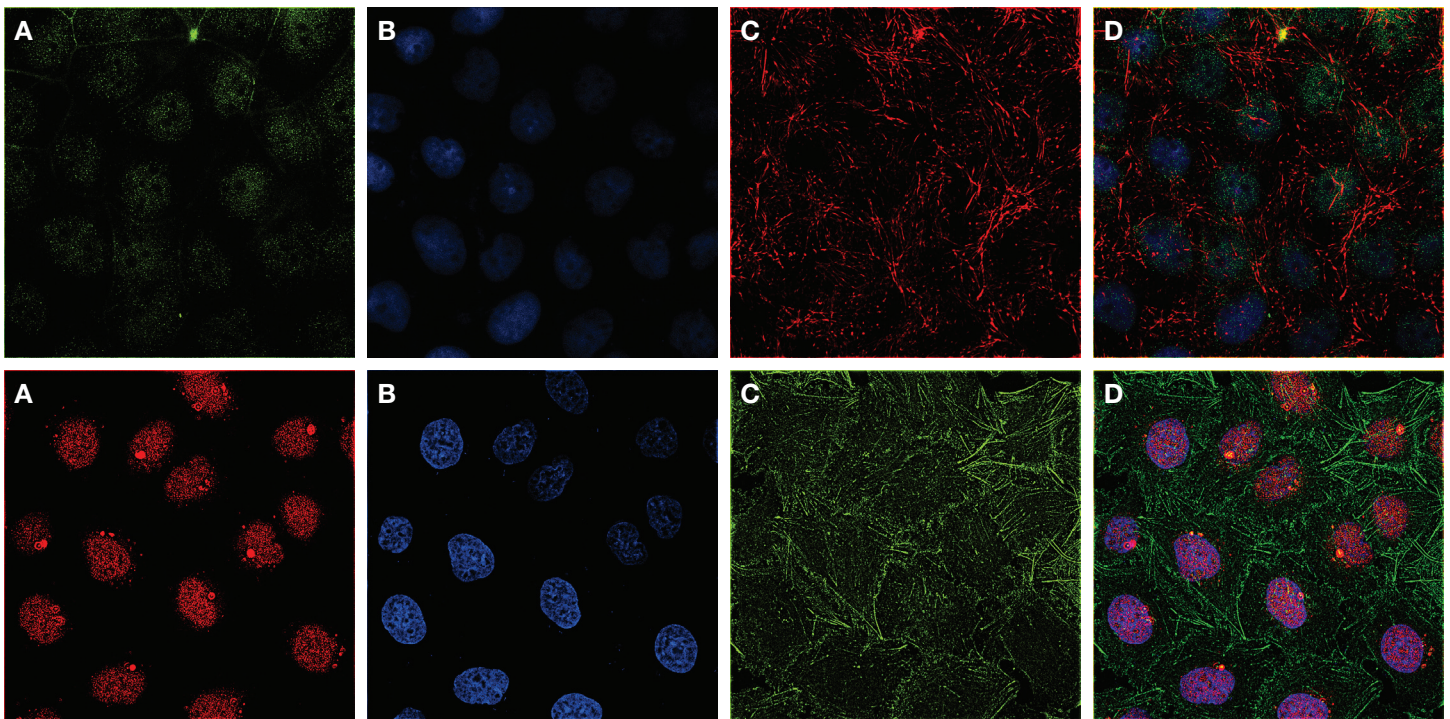


Figure 3. Nuclear marker expression. Top row: 70% confluent log-phase Caco-2 cells were stained with Invitrogen™ TBX5 Polyclonal Antibody (Cat. No. 42-6500). Bottom row: 70% confluent log-phase HeLa cells were stained with Invitrogen™ RANBP3 Recombinant Monoclonal Antibody (Cat. No. 700076). (A) Protein of interest, (B) DNA stained with DAPI, (C) F-actin stained with Invitrogen™ Alexa Fluor™ 488 phalloidin or rhodamine phalloidin, (D) merged image.

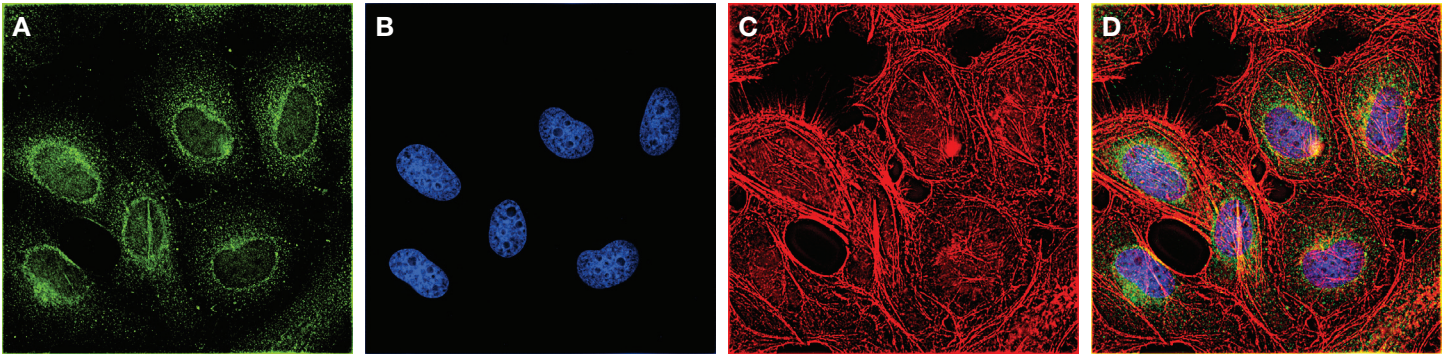


Figure 4. Perinuclear and nucleolar marker expression. 70% confluent log-phase HeLa cells were stained with Invitrogen™ VRK1 Monoclonal Antibody (Cat. No. MA1-828). **(A)** Protein of interest, **(B)** DNA stained with DAPI, **(C)** F-actin stained with rhodamine phalloidin, **(D)** merged image.

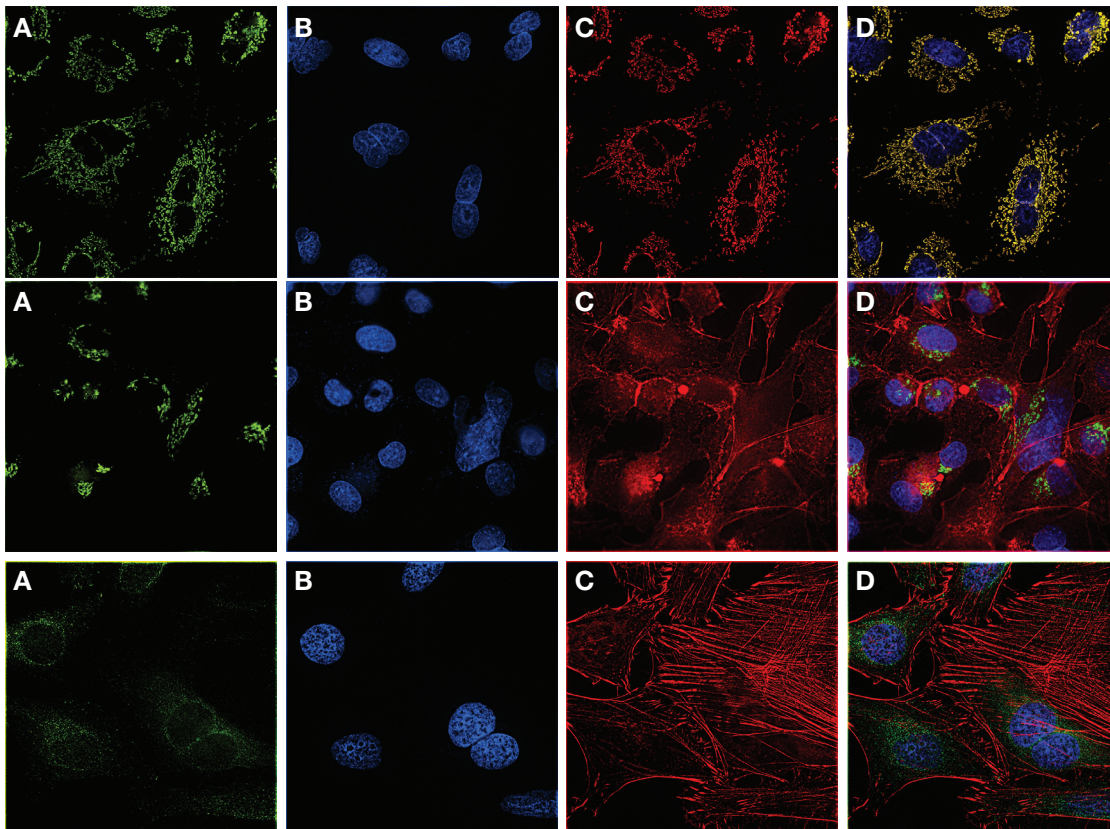


Figure 5. Intracellular organelle marker expression. **Top row:** 70% confluent log-phase A549 cells were stained with Invitrogen™ Mitochondria Monoclonal Antibody (Cat. No. MA5-12014). **(A)** Protein of interest, **(B)** DNA stained with DAPI, **(C)** mitochondria stained with Invitrogen™ MitoTracker™ Red CMXRos (Cat. No. M7512), **(D)** merged image. **Middle row:** 70% confluent log-phase HepG2 cells were stained with Invitrogen™ TGN46 Polyclonal Antibody (Cat. No. PA1-1069). **(A)** Protein of interest, **(B)** DNA stained with DAPI, **(C)** F-actin stained with rhodamine phalloidin, **(D)** merged image. **Bottom row:** 70% confluent log-phase HeLa cells were stained with Invitrogen™ RAB11B Polyclonal Antibody (Cat. No. PA5-31348). **(A)** Protein of interest, **(B)** DNA stained with DAPI, **(C)** F-actin stained with rhodamine phalloidin, **(D)** merged image.

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