Ki67 Immunofluorescence on Bovine Cell Lines

Justine Marsolier, Souhila Medjkane, Martine Perichon and Jonathan B. Weitzman*

Epigenetics and Cell Fate, UMR 7216 CNRS, Université Paris Diderot, Sorbonne Paris Cité, Paris, France

*For correspondence: jonathan.weitzman@univ-paris-diderot.fr

[Abstract] This is a rapid protocol to test the effects of drugs treatment on bovine cell replication using Ki67 staining. Ki67 is associated with cell proliferation and is present during all active phases of the cell cycle (G1, S, G2, and mitosis), but is absent from resting cells (G0).

Materials and Reagents

1. Bovine cells (The TBL3 cell line was derived from *in vitro* infection of the spontaneous bovine-B lymphosarcoma cell line, BL3, with Hissar stock of *T. annulata*)
2. RPMI 1640 (Gibco)
3. Fetal calf serum (heat-inactivated)
4. Penicillin/streptomycin
5. Fibronectin (Sigma-Aldrich, catalog number: F1141)
6. Formaldehyde (Sigma-Aldrich, catalog number: F8775)
7. Triton X-100 (Sigma-Aldrich, catalog number: T9284)
8. SVF (PAA Laboratories GmbH, catalog number: A15-101)
9. BSA (Sigma-Aldrich, catalog number: A2153)
10. Mouse monoclonal anti-Ki67 (1:50) (Abcam, catalog number: ab10913-1)
11. Cy2 AffinyPure anti-mouse IgG (1:5,000) (Jackson ImmunoResearch Laboratories, catalog number: 715-225-150)
12. Tween 20 (Biosolve, catalog number: 2045 2335)
13. ProLong Gold Antifade Reagent with Dapi (Life Technologies, Invitrogen™, catalog number: P-36931)
14. DAPI

Equipment

1. Slides (Knittel Glass, catalog number: KN00010025787)
2. 37 °C, 5% CO_2 cell culture incubator
3. 24 well plate

4. Fluorescent microscope (Leica Microsystems, model: Inverted 6000)

Procedure

1. Bovine cells were cultured in RPMI 1640, supplemented with 10% heat-inactivated Fetal calf serum, 4 mM L-Glutamine, 25 mM HEPES, 10 µM β-mercaptoethanol and 100 µg/ml penicillin/streptomycin in a humidified 5% CO_2 atmosphere at 37 °C.
2. In 24 wells plate, slides were coated with PBS – Fibronectin (1/1,000), 2 h at 37 °C and wash twice with PBS.
3. Non adherent bovine cells (500,000 cells/well in 24 wells plate) were plated on Fibronectin coated slides 2 h at 37 °C, 5% CO_2 and then fixed in 500 µl PBS 3.7% Formaldehyde for 15 min at room temperature.
4. Slides were rinsed in PBS (300 µl - 3 washes) and permeabilized with PBS 0.2% Triton X-100 for 5 min.
5. Slides were washed in PBS (300 µl - 3 washes) and then blocked for 30 min at room temperature with 250 µl PBS 1% SVF and 1% BSA to prevent non-specific staining.
6. The slides were incubated with Mouse monoclonal anti-Ki67 (1:50) in 100 µl PBS 1% SVF and 1% BSA at room temperature for 40 min.
7. After washing in PBS 0.2% Tween (300 µl - 3 washes), the slides were incubated with Cy2 AffinyPure anti-mouse IgG (1:5,000) in 100 µl PBS 1% SVF and 1% BSA for 30 min at room temperature, in the dark.
8. Slides were subsequently washed in PBS 0.2% Tween (300 µl - 3 washes), mounted on slides and coverslippedwith ProLong Gold Antifade Reagent with DAPI.
9. Images of immunofluorescence staining were photographed with a camera attached to a fluorescent microscope and percentage of Ki67 positive cells was calculated.
10. This staining was repeated for three independent biological replicates (Figure 1).

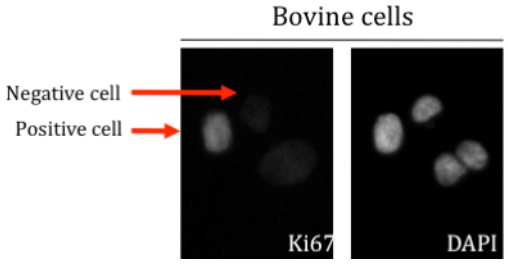

Figure 1. Ki67 staining of bovine cells

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References

1. James, D., Levine, A. J., Besser, D. and Hemmati-Brivanlou, A. (2005). TGFbeta/activin/nodal signaling is necessary for the maintenance of pluripotency in human embryonic stem cells. *Development* 132(6): 1273-1282.
2. Marsolier, J., Pineau, S., Medjkane, S., Perichon, M., Yin, Q., Flemington, E., Weitzman, M. D. and Weitzman, J. B. (2013). OncomiR addiction is generated by a miR-155 feedback loop in *Theileria*-transformed leukocytes. *PLoS Pathog* 9(4): e1003222.

