IFN-α Inhibition Assay *in vitro*

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[Abstract] During viral infections Interferon-α (IFN-α) is expressed by infected host cells. IFN-α binds to its receptor (IFNAR1/2), which leads to the activation of downstream signaling via JAK-STAT. This signaling cascade results in the expression of several hundred different genes, so called interferon-stimulated gene, which lead to an antiviral state of the infected and the neighboring cells.

Materials and Reagents

1. *Mus dunni* fibroblast cells
2. Friend murine leukemia virus (F-MuLV) (titer was measured as previously described (Robertson *et al.*, 1991))
3. 3-Amino-9-ethylcarbazole (AEC) (Sigma-Aldrich, catalog number: A6926-100TAB)
4. Antibody 720 (mouse antibody against FV envelope protein), hybridoma supernatant (Robertson *et al.*, 1991)
5. Bovine serum albumin (BSA) (PAA Laboratories GmbH, catalog number: K41-001)
6. Ethanol 96% (Roth North America, catalog number: 5054.5)
7. Superior FBS (fetal bovine serum, not heat-inactivated) (Biochrom, catalog number: S0615)
8. Goat anti-mouse HRP (Dako, catalog number: P0477)
9. Hydrogen peroxide 30% (Applichem, catalog number: A1134,0250)
10. N,N-dimethylformamide (Merck Millipore, catalog number: 1.03053.1000)
11. PBS (GIBCO, catalog number: 14190-136)
12. Penicillin/streptomycin (PAA Laboratories GmbH, catalog number: P11-010)
13. Polybrene/Hexadimethrine bromide (Sigma-Aldrich, catalog number: H9268)
14. RPMI 1640 (PAA Laboratories GmbH, catalog number: E15-840)
15. Sodium Acetate (Merck Millipore, catalog number: 1062811000)
16. Murine IFN-α (PBL, catalog number: 12100-1)
17. Medium (see Recipes)
18. Washing buffer (see Recipes)
19. 3-Amino-9-ethylcarbazole (AEC) substrate solution (see Recipes)

Equipment

1. Incubator (37 °C; 5% CO_2)
2. 24 well cell culture plate (Greiner Bio-one, catalog number: 662160)

Procedure

Day 1:

1. Seed 7.5 x 10^3 *Mus dunni* fibroblast cells in 500 µl media per well in 24-well plates.
2. Add IFN-α in increasing concentrations to the cells (use concentrations between 50 pg/ml to 10,000 pg/ml).
3. Controls: Without IFN-α; without virus.
4. Incubate the cells for 24 h at 37 °C (5% CO_2).

Day 2:

1. Decant media.
2. Add 1 ml fresh media supplemented with polybrene (8 µg/ml) to increase the infection efficiency.
3. Add 50 FFU (focus-forming units) F-MuLV to the wells.
4. Incubate for 3 days.

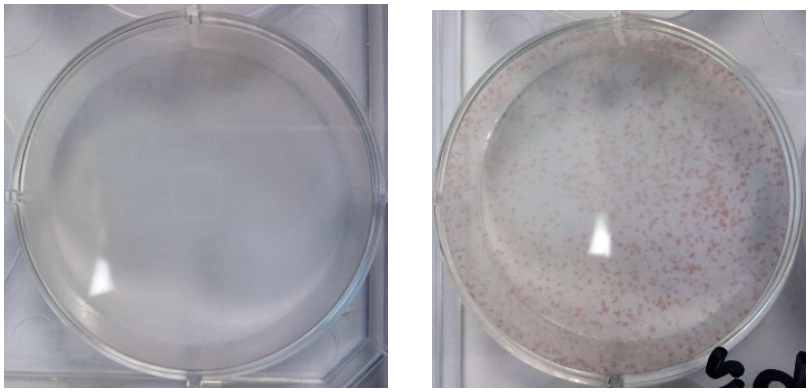
Day 5:

1. Decant media.
2. Fix cells with 500 µl 96% ethanol for 5 min at room temperature (RT).
3. Wash wells twice with 500 µl washing buffer (PBS + 0.1% BSA).
4. Add 250 µl supernatant of antibody 720 (mAB against FVenv) per well for 2 h.
5. Wash twice with 500 µl washing buffer.
6. Add 250 µl 2nd antibody (goat anti-mouse HRP) to wells (diluted 1 to 500 in PBS).
7. Incubate for 1 h at RT.
8. Wash twice with 500 µl washing buffer.
9. Freshly prepare AEC substrate solution as indicated in Recipes section.
10. Add 250 µl substrate solution per well.
11. Incubate for 10-15 min at RT in the dark.
12. Decant supernatant in special waste container for toxic solvents.
13. Wash with 500 µl water.
14. Dry plates overnight.

Day 6:

1. Count foci

   Treatment with IFN-α should significantly decrease the numbers of foci compared to unstimulated cells (Figure 1).



without foci with foci

Figure 1. Representative example of Interferon-α Inhibition assay with Interferon-α (left picture without foci) and without Interferon-α (right picture with foci)

Recipes

1. Medium

   RPMI 1640

   10% FCS

   1% penicillin/streptomycin
2. Washing buffer

   PBS + 0.1% BSA
3. AEC substrate solution

   Dissolve 1 tablet AEC in 2.5 ml of *N*, *N*-dimethylformamide. Add 2.5 ml of the substrate solution to 47.5 ml of 50 mM sodium acetate buffer, pH 5.0. Add 25 µl of fresh 30% hydrogen peroxide immediately prior to use.

Acknowledgments

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