IFN- α Inhibition Assay *in vitro*

Kathrin Gibbert*

Department of Virology, University of Duisburg-Essen, Essen, Germany

*For correspondence: Kathrin.gibbert@uni-due.de

[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% CO₂)
2. 24 well cell culture plate (Greiner Bio-one, catalog number: 662160)

Procedure

Day 1:

1. Seed 7.5×10^3 *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% CO₂).

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).

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 μ l of fresh 30% hydrogen peroxide immediately prior to use.

Acknowledgments

This work was supported by the Deutsche Forschungsgemeinschaft (GRK 1045).

References

1. Dittmer, U., Brooks, D. M. and Hasenkrug, K. J. (1998). [Characterization of a live-attenuated retroviral vaccine demonstrates protection via immune mechanisms.](#) *J Virol* 72(8): 6554-6558.
2. Gerlach, N., Gibbert, K., Alter, C., Nair, S., Zelinsky, G., James, C. M. and Dittmer, U. (2009). [Anti-retroviral effects of type I IFN subtypes *in vivo*.](#) *Eur J Immunol* 39(1): 136-146.
3. Gibbert, K., Joedicke, J. J., Meryk, A., Trilling, M., Francois, S., Duppach, J., Kraft, A., Lang, K. S. and Dittmer, U. (2012). [Interferon-alpha subtype 11 activates NK cells and enables control of retroviral infection.](#) *PLoS Pathog* 8(8): e1002868.
4. Robertson, M. N., Miyazawa, M., Mori, S., Caughey, B., Evans, L. H., Hayes, S. F. and Chesebro, B. (1991). [Production of monoclonal antibodies reactive with a denatured form of the Friend murine leukemia virus gp70 envelope protein: use in a focal infectivity assay, immunohistochemical studies, electron microscopy and western blotting.](#) *J Virol Methods* 34(3): 255-271.