Measurement of IFN-α Subtype Concentrations (Virus-free, Cell-based Bioassay)

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[Abstract] The induction of type I IFN is the immediate host response against viral infections. Type I IFNs belong to a multigene family including up to 14 different IFN-α subtypes and one IFN-β. They are highly conserved and bind the same receptor (IFNAR1/2) with varying affinities, although they differ in their biological activities.

Materials and Reagents

1. 7AAD (7-amino-actinomycin D) (BD Pharmingen, catalog number: 51-68981E)
2. Bovine serum albumin (BSA) (PAA Laboratories GmbH, catalog number: K41-001)
3. DMEM (Life Technologies, Gibco®, catalog number: 41966-029)
4. Superior FBS (fetal bovine serum, not heat-inactivated) (Biochrom, catalog number: S0615)
5. Mx/RAGE7 cells (virus-transformed adherent cell line with a temperature-inducible promotor; must be cultured at 32 °C; cells express the Mx transgene and a promotorless eGFP gene which is expressed due to type I IFN stimulation) (Bollati-Fogolin and Muller, 2005)
6. PBS (Life Technologies, Gibco®, catalog number: 14190-136)
7. Penicillin/streptomycin (PAA Laboratories GmbH, catalog number: P11-010)
8. Propidium iodide (eBioscience, catalog number: 00-6990-50)
9. Murine IFN-α (PBL, catalog number: 12100-1)
10. Sodium azide (Applichem, catalog number: A1430.0010)
11. Sodium pyruvate (Life Technologies, Gibco®, catalog number: 11360-039)
12. Trypsin EDTA (PAA Laboratories GmbH, catalog number: L11-004)
13. β-mercaptoethanol (Life Technologies, Gibco®, catalog number: 31350-010)
14. Media for Mx/RAGE7 cells (see Recipes)
15. FACS buffer (see Recipes)

Equipment

1. 96-well flat bottom plate (Falcon BD Labware, catalog number: 3072)

2. 1.5 ml microfuge tubes
3. FACS tubes (BD Biosciences, Falcon®, catalog number: 352054)
4. Flow cytometer (*e.g.* BD LSR II)
5. Incubator (37 °C; 5% CO_2)
6. Incubator (32 °C; 5% CO_2)

Procedure

Different murine IFN-α subtypes (IFN-α1, -α2, -α4, -α5, -α6, -α9, -α11) were produced as already described (Gerlach *et al*., 2009).

Day 1:

1. Seed Mx/RAGE7 cells in a 96 well cell culture plate (2 x 10^4 cells per well in 200 µl medium).
2. Grow the cells for 24 h at 32 °C.

Day 2:

1. Perform serial dilutions (log10) of produced IFN-α subtypes in medium in 1.5 ml tubes.
2. Perform serial dilutions (log2) of recombinant IFN-α subtypes (PBL) with known concentrations from 1,000 U/ml to 31.25 U/ml (= standards) in 1.5 ml tubes.
3. Decant medium of Mx/RAGE7 cells.
4. Add 200 µl of the IFN-α solutions with known (standards) and unknown concentrations to the cells.
5. As negative control add 200 µl of medium without IFN-α.
6. Incubate the samples for 24 h at 37 °C.

Day 3:

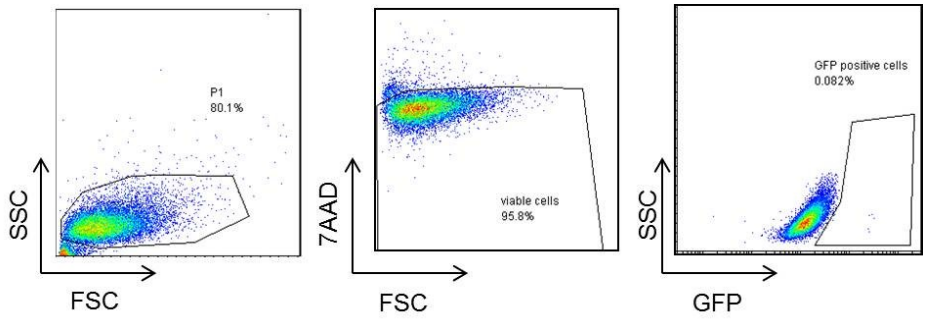
1. Decant the medium.
2. Add 200 µl fresh medium to the cells.
3. Incubate the samples for 48 h at 37 °C.

Day 5:

1. Decant the medium.
2. Wash cells with 200 µl PBS.
3. Add 50 µl of trypsin EDTA (1x) 0.05% to the cells at room temperature until they suspend.
4. Harvest suspended cells in FACS tubes containing 1 ml of PBS.
5. Centrifuge cells (300 *x g*; 5 min).

6. Resuspend cells with 250 µl FACS buffer.
7. Add 2.5 µl 7AAD or 0.5 µl propidium iodide per sample to exclude dead cells.
8. Immediately analyze cells with flow cytometer.
9. IFN-α treated Mx/RAGE7 cells express eGFP (Figure 1).

Without IFN-α



Stimulated with IFN-α

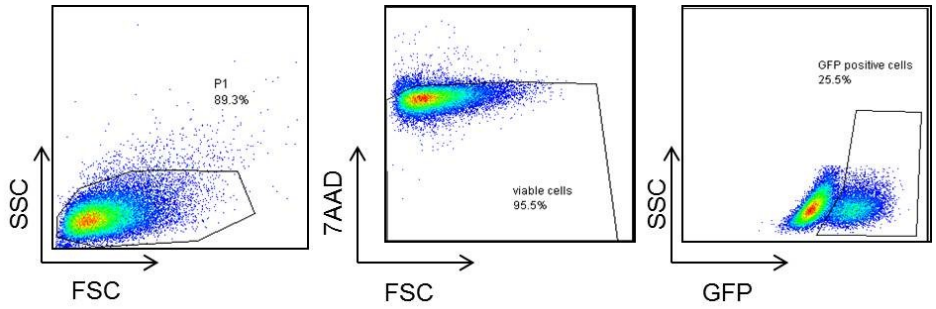


Figure 1. Representative dot plots of Mx/RAGE7 cells without IFN-α (upper panel) and with IFN-α (lower panel)

10. Perform standard curve with samples treated with known IFN-α concentrations (graph the data for the standard curve (Figure 2), the IFN-α titer can be determined by comparison).
11. Calculate concentrations of unknown samples.

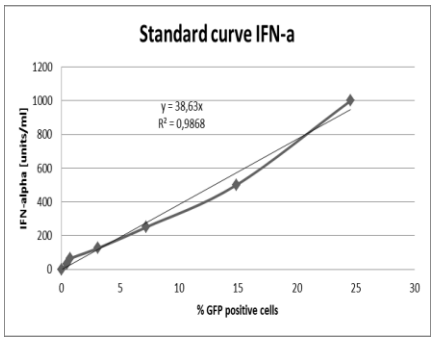


Figure 2. Standard curve of IFN-α

Recipes

1. Media for Mx/RAGE7 cells
   DMEM
   10% FBS
   1 mM sodium pyruvate
   1% penicillin/streptomycin
   50 µM β-mercaptoethanol
2. FACS buffer
   PBS
   0.1% BSA
   0.02% sodium azide

Acknowledgments

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References

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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.