

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% CO₂)
6. Incubator (32 °C; 5% CO₂)

Procedure

Different murine IFN- α subtypes (IFN- α 1, - α 2, - α 4, - α 5, - α 6, - α 9, - α 11) 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 10⁴ cells per well in 200 μ l 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 μ l of the IFN- α solutions with known (standards) and unknown concentrations to the cells.
5. As negative control add 200 μ l of medium without IFN- α .
6. Incubate the samples for 24 h at 37 °C.

Day 3:

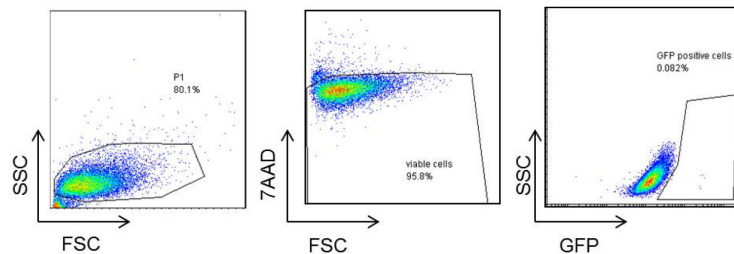
1. Decant the medium.
2. Add 200 μ l fresh medium to the cells.
3. Incubate the samples for 48 h at 37 °C.

Day 5:

1. Decant the medium.
2. Wash cells with 200 μ l PBS.
3. Add 50 μ l 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 μ l FACS buffer.
7. Add 2.5 μ l 7AAD or 0.5 μ l 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).
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.

Without IFN- α



Stimulated with IFN- α

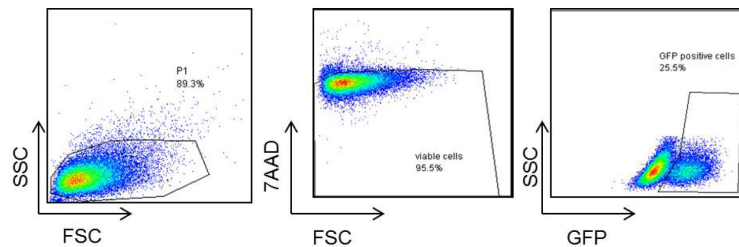


Figure 1. Representative dot plots of Mx/RAGE7 cells without IFN- α (upper panel) and with IFN- α (lower panel)

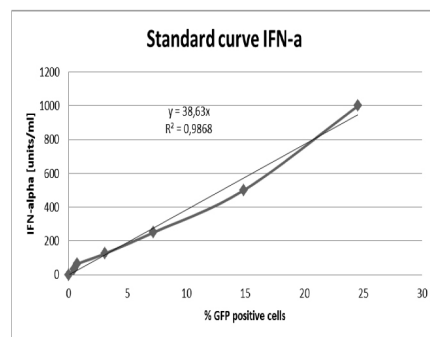


Figure 2. Standard curve of IFN- α

Recipes

1. Media for Mx/RAGE7 cells

- DMEM
- 10% FBS
- 1 mM sodium pyruvate
- 1% penicillin/streptomycin
- 50 μ M β -mercaptoethanol
- 2. FACS buffer
- PBS
- 0.1% BSA
- 0.02% sodium azide

Acknowledgments

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References

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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.
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