



Certificate of Analysis

Rhesus/Cynomolgus Interferon Alpha 2

Catalog No: 14110-9

Lot No: 6572

Size: 1 mg/vial

Description: Recombinant Rhesus/Cynomolgus Interferon Alpha 2 (Rhesus/Cynomolgus IFN-α2), carrier free

Volume: 3.077 ml

Activity: 3.32 x 10<sup>7</sup> units/ml (MDBK/VSV)

Specific Activity: 1.02 x 10<sup>8</sup> units/mg (MDBK/VSV)

Buffer: 25 mM HEPES, pH 5; 6% Glycerol; 0.7M Sodium Chloride

Endotoxin: < 1 EU/μg

Molecular Weight: 19.5 kDa

Purity: > 98%

Source: Rhesus cDNA expressed in E. coli

Accession #: XM\_001107516

Assay Used to Measure Bioactivity: Interferon was titrated with the use of the cytopathic effect inhibition assay as listed. The protein has similar activity to Human IFN Alpha 2 on human, rhesus and bovine cells.

Bovine (MDBK/VSV) – performed as described [Rubinstein et al. (1981) J. Virol. 37(2):755] The EC<sub>50</sub> for interferon in this assay is ~5 U/ml.

Human (A549/EMCV) – performed as described [Budd et al. (1985) Canc. Chem. Pharm. 12:39]. The EC<sub>50</sub> for interferon in this assay is ~1 U/ml.

Rhesus (LLC-MK2/VSV) – Comparative studies using LLC-MK2 cells versus A549 cells have shown that the activity of the protein on A549 cells is indicative of its activity on LLC-MK2 cells.

As there is no international Monkey Interferon Alpha standard, the units are determined by use of a Human IFN Alpha A (Hu-IFN-αA [2a]) laboratory standard calibrated to the international reference standard for Human Interferon Alpha A (Hu-IFN-αA [2a]) provided by the National Institutes of Health [Meager et al. (2001). J Immunol. Methods 257(1-2):17-33].

Product Information: The cDNA for Rhesus/Cynomolgus IFN-α2 was cloned from the Rhesus cell line LLC-MK2. This was found to be identical to the putative Rhesus IFN-α2 (XM\_001107516) identified as part of the Rhesus genome project (http://www.hgsc.bcm.tmc.edu/projects/rmacaque). There are 14 AA differences in the mature protein (2 in the signal peptide) between the Rhesus and Human Alpha A (2a) sequence. There are 13 AA differences in the mature protein (2 in the signal peptide) between the Rhesus and Human Alpha 2 (2b) sequence. This is in accord with a previous report [Villinger et al. (1995) J. Immunol. 155:3946] stating that there are 15-16 amino acids different between the Rhesus IFN alpha 2 and human alpha 2 (2b) and human alpha A(2a) respectively.

This Rhesus (Macaca mulatta) sequence is also identical to a Cynomolgus (Macaca fascicularis) IFN-α2 cloned from cDNA isolated from primary hepatocytes (PBL unpublished observation).

Comparison of the Mature Sequences of Human IFN Alpha 2b and Rhesus IFN Alpha 2

Table with 2 columns: Label (Rhesus/Human) and Position (1/61). It shows amino acid sequences for Rhesus and Human IFN Alpha 2, highlighting differences with dots and bold letters.



Rhesus 121 KYFQRITLYL KEKKYSLCAW EVVRAEIMRS FSLSTNLQES LRSKE
Human 121 .....P.....

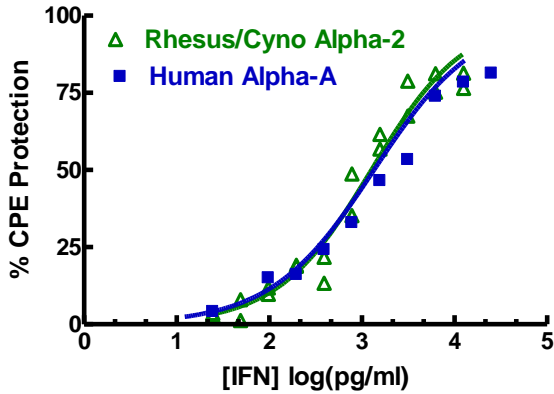


Figure 1: Activity of Rhesus/Cyno and Human IFN Alpha A on rhesus cells (LLC-MK2) challenged with VSV.

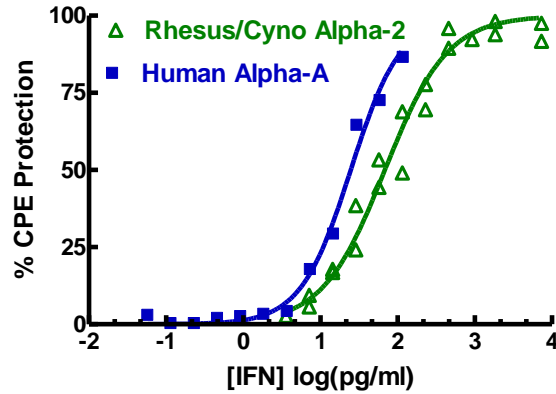


Figure 2: Activity of Rhesus/Cyno and Human IFN Alpha A on human cells (A549) challenged with EMCV.

Results are representative and may vary depending upon experimental conditions.

Shipping Conditions: Dry Ice

Physical State of Product During Shipping: Frozen

Storage Conditions/Comments: After receipt, the product may be stored at -20°C for short-term use (≤ 6 months). For long-term storage, we recommend storing the product at -70°C or below for retention of full activity. When thawing, the contents of the tube should be apportioned in separate tubes so that freezing and thawing is kept to a minimum. Refreezing should be done on dry ice or in a dry ice/alcohol bath. Further dilution of the product should be in buffers containing protein such as 0.1% bovine serum albumin (BSA). For more information on protein handling, visit our Resource Library at www.pbl assaysci.com.

Authorization

Released by: [Signature]

Date: October 6, 2016

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