



Certificate of Analysis

Rhesus/Cynomolgus Interferon Alpha 2

Catalog No: 14110-1

Lot No:

Size: ≥ 1 x 10⁵ units/vial

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

Volume: 0.1 ml

Activity: x 10 units/ml (MDBK/VSV)

Specific Activity: x 10 units/mg (MDBK/VSV)

Buffer: Phosphate buffered saline (PBS) containing 0.1% bovine serum albumin (BSA)

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 EC₅₀ 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 EC₅₀ for interferon in this assay is ~1 U/ml. Activity: x 10 units/ml Specific Activity: x 10 units/mg.

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

Rhesus 1	CDLPQTHSLG	NRRTLMLLAQ	MRRISLFFCL	KDRHDFEFPQ	EEFGNQFQKA	QTIPVLHEMI
Human 1S.....S.....G....E.....		



Rhesus 61	QQTFNLFSTK	DSSAAWDETL	LNKFYTELYQ	QLNDLEACVM	QEMGVTETPL	MNKNSILAVR
Human 61	..I.....D.....I	.GV.....	.KED.....
Rhesus 121	KYFQRITLYL	KEKKYSLCAW	EVVRAEIMRS	FSLSTNLQES	LRKE	
Human 121P.....	

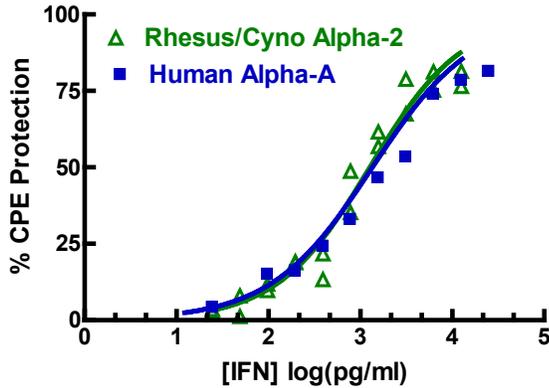


Figure 1: Activity of Rhesus/Cyno and Human IFN Alpha A on rhesus cells (LLC-MK2) challenged with VSV.

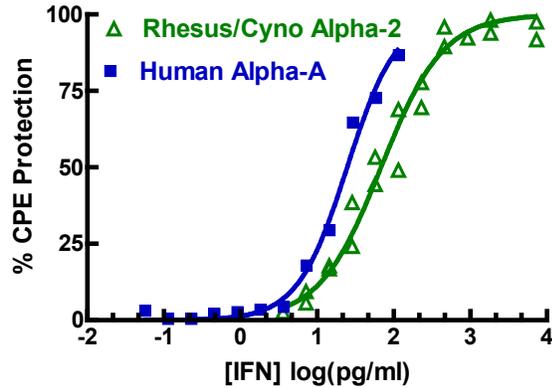


Figure 2: Activity of Rhesus/Cyno and Human IFN Alpha A on human cells (A549) challenged with EMCV.

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: _____

Date:

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