

## A simple, scalable and high yield method for high level production of human recombinant interferon

### SUMMARY

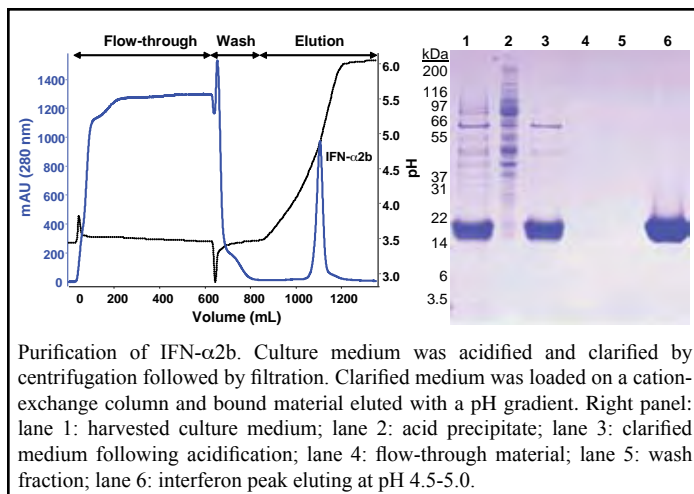
Interferons are cytokines with major current and potential therapeutic applications. To date, the production of recombinant cytokines in mammalian systems has not been well explored due to limitations in the volumetric productivity. The present technology provides a stable clone derived from the HEK293 mammalian cell line that produces high levels of biologically active human glycosylated interferon alpha-2b (IFN- $\alpha$ 2b), and a rapid procedure for its efficient recovery in high yields and purity. Together, they allow the efficient manufacturing of a more potent IFN- $\alpha$ 2b than its non-glycosylated form produced by *Escherichia coli*.

### APPLICATIONS

- High volumetric production and purification of glycosylated human recombinant IFN- $\alpha$ 2b.
- Potentially, large-scale production of other interferons and cytokines.

### CONCEPT

The antiviral, antiproliferative, and immunomodulatory activities exhibited by interferons have led to their use as therapeutic agents. Interferon alpha-2b (IFN- $\alpha$ 2b) is the predominant subvariant of interferons. Some of the many diseases treated with IFN- $\alpha$ 2b, alone or in combination, include type B and C hepatitis, and several cancers. Human recombinant IFN- $\alpha$ 2b used in the clinic is currently synthesized in bacterial systems. A major inconvenient of these systems is the requirement for a refolding step to restore recombinant IFN- $\alpha$ 2b to its correct and active 3-D structure and a pegylation step to alleviate the negative impacts associated with a non-glycosylated form of the protein. These extra processes not only reduce recovery rates (typically <20%), but they may lower specific activity and may trigger an immune response. To circumvent the barriers of bacterial expression systems, a mammalian HEK293 cell line constitutively expressing the EBNA1 protein of EBV (clone 6E) was used in combination with a plasmid derivative of the pTT vector encoding the human IFN- $\alpha$ 2b gene to generate IFN-producing



Purification of IFN- $\alpha$ 2b. Culture medium was acidified and clarified by centrifugation followed by filtration. Clarified medium was loaded on a cation-exchange column and bound material eluted with a pH gradient. Right panel: lane 1: harvested culture medium; lane 2: acid precipitate; lane 3: clarified medium following acidification; lane 4: flow-through material; lane 5: wash fraction; lane 6: interferon peak eluting at pH 4.5-5.0.

clones (NRC no. 11565 & 11266). One clone that stably produced high IFN- $\alpha$ 2b levels while maintaining a high growth rate was selected. This clone was shown to produce biologically active IFN- $\alpha$ 2b with a gene reporter assay. Further, a purification process allowing high purity and recovery was also developed. Together, these results demonstrate the ability to cost-effectively produce and purify potent glycosylated IFN- $\alpha$ 2b from human cells.

### FEATURES AND BENEFITS

#### Important market opportunities

The global market for interferon alpha is currently estimated at about US\$2.5 billion. The rising incidence of certain cancers and viral hepatitis, in addition to ongoing investigations of novel therapeutic applications are increasing the needs for human recombinant IFN- $\alpha$ 2b. There are also significant opportunities for growth in the generics market as IFN- $\alpha$ 2b recently reached the end of its patent protection.

#### Superior production and recovery of IFN- $\alpha$ 2b

The volumetric productivity and recovery of HEK293-produced IFN- $\alpha$ 2b is comparable to some productions of non-glycosylated IFN- $\alpha$ 2b performed in *E. coli* and in the methylotrophic yeast *Pichia pastoris*. The volumetric production of IFN- $\alpha$ 2b with the selected HEK293 clone largely and reproducibly exceeded 200 mg/L in a simple fed-batch serum-free culture maintained for only 7-8 days. The simple, rapid and cost-effective method developed for the recovery of HEK293-produced IFN- $\alpha$ 2b yielded 98% pure interferon with a recovery greater than 75%. In addition, IFN- $\alpha$ 2b productivity of this clone remained stable in the absence of selection pressure for more than nine months in culture.

#### Proper post-translational modifications and good biologic activity

One of the major therapeutic interests for producing IFN- $\alpha$ 2b in mammalian cells is to generate a glycosylated protein with high biological activity. The IFN- $\alpha$ 2b produced in HEK293 cells is 0-glycosylated and extensively sialylated. The O-glycosylation is similar to that exhibited by IFN- $\alpha$ 2b produced by human peripheral blood leucocytes. Further, the signal peptide was shown to be correctly processed and the protein to be at least as active as bacterially produced IFN- $\alpha$ 2b using a reporter gene assay.

#### Suitable host for the large-scale production of other interferons and cytokines

With the exception of the aforementioned HEK293 cell clone, recombinant cytokines are typically produced in much lower quantities in mammalian expression systems. Accordingly, the adaptability of HEK293 cells to a growth inhibitory cytokine suggests that they may be suitable hosts for the large-scale production of other interferons and cytokines.

### PROTECTION STATUS

Production and purification of recombinant interferon proteins (NRC no. 11993).

### CONTACTS

#### Yves Quenneville

Tel.: (514) 496-8507

Business Development Officer

E-mail: yves.quenneville@cnrc-nrc.gc.ca

#### Daniel Desmarteaux

Tel.: (514) 496-5300

Business Development Officer

E-mail: daniel.desmarteaux@cnrc-nrc.gc.ca

#### Dr. Yves Durocher

Tel.: (514) 496-6192

Animal Cell Technology Group

E-mail: yves.durocher@cnrc-nrc.gc.ca