

Application Note

CellPrime™ Recombinant Human Transferrin as an Animal-Free Alternative to Animal-Derived Transferrin or Iron Supplementation in Cell Culture Applications

INTRODUCTION

Iron is essential for key cellular processes, including cell growth and DNA synthesis. Iron is typically transported *in vivo* by transferrin, which regulates efficient delivery of iron to cells. In the biopharmaceutical setting, antibody-producing cell lines such as CHO and NS0 require an iron source such as transferrin to attain optimal cell growth and protein production. Traditionally transferrin has been supplied in the form of serum-derived purified human transferrin (hTf) or bovine transferrin (bTf). Current regulatory pressure to remove animal-derived products from biopharmaceutical manufacturing has led researchers to explore alternate means to animal-derived transferrin. As an alternative, inorganic iron salts have been used to supply iron to mammalian cells. However, to provide high density cell cultures with sufficient iron, elevated concentrations of iron salts are required that utilize low affinity

non-transferrin receptor pathways. This can negatively impact cell growth due to the formation of free radicals and oxidative stress from the unbound ferric or ferrous irons.

CellPrime rTransferrin (Millipore Corporation) is a recombinant human transferrin that provides an animal-free alternative to iron salts for industrial cell culture. CellPrime rTransferrin is a recombinant analogue of human transferrin expressed in *Saccharomyces cerevisiae*. Supplied as a human holo-transferrin analogue, CellPrime rTransferrin binds specifically to the transferrin receptor, thereby facilitating iron uptake into the cell for optimal cell culture performance. In this paper, we describe cell culture data demonstrating that CellPrime rTransferrin shows equivalence to hTf and superiority to bTf in stimulating cell growth and protein production across a number of industrially-relevant cell lines.

MATERIALS AND METHODS

Cell Line Culture and Maintenance

CHODUKXB11 clones expressing human mAbs against human interleukin (IL)-8 and the beta chain of CD-18 (referred to as CHO DP-12 and CHO B13-24 respectively) were cultured in DMEM/F12, 10% FBS, 4.5 g/L glucose, 2 mg/L recombinant human insulin, 200 nM MTX and 2 mM Glutamax™ in T25 flasks. Prior to assay set up, cells were grown to between 70 and 90% confluence in T75 tissue culture flasks in 10–20 mL sterile culture media (as above), spun and detached using trypsin. Cells were then washed in 10 mL Dulbecco's PBS, spun and resuspended in serum-free, growth factor free medium and cell viability determined by the trypan blue exclusion method.

96-well Growth and Productivity Assay

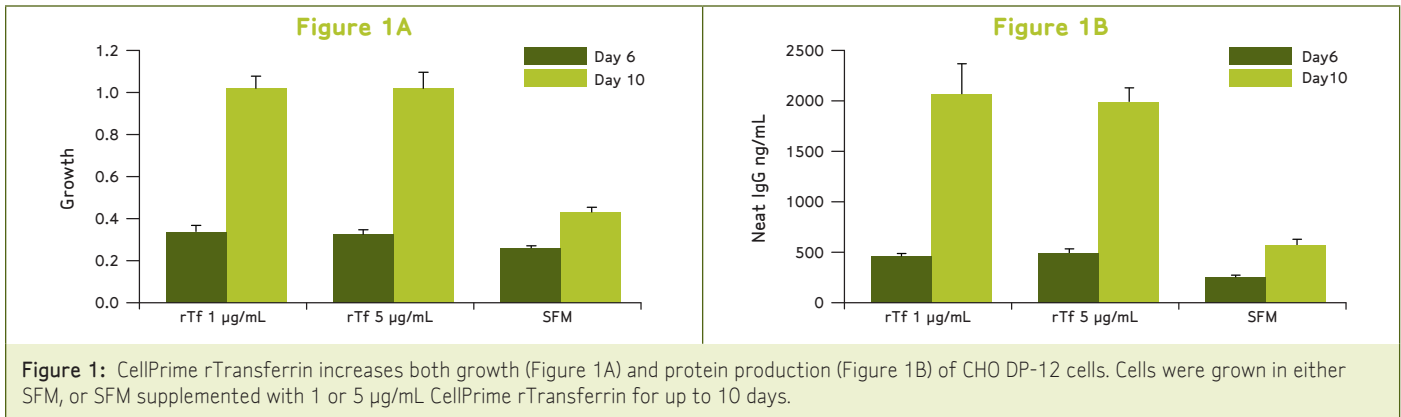
1000 cells in fresh SFM were added to each well of a 96 well plate. All supplements and controls were tested in 6 replicate wells. Plates were incubated at 37 °C/5% CO₂ and harvested at either day 6 or 10. On day of harvest, 100 µl of conditioned media was removed to a separate plate for IgG analysis. The metabolic dye MTS was added to the remaining media as an indication of viable cell numbers and absorbance measured to determine growth. Cell growth was measured from each of the 6 replicate wells and pairs of wells were pooled to create 3 replicates of pooled samples for IgG analysis by ELISA.

RESULTS

CellPrime rTransferrin Increases Growth and Protein Production in Two CHO Cell Lines

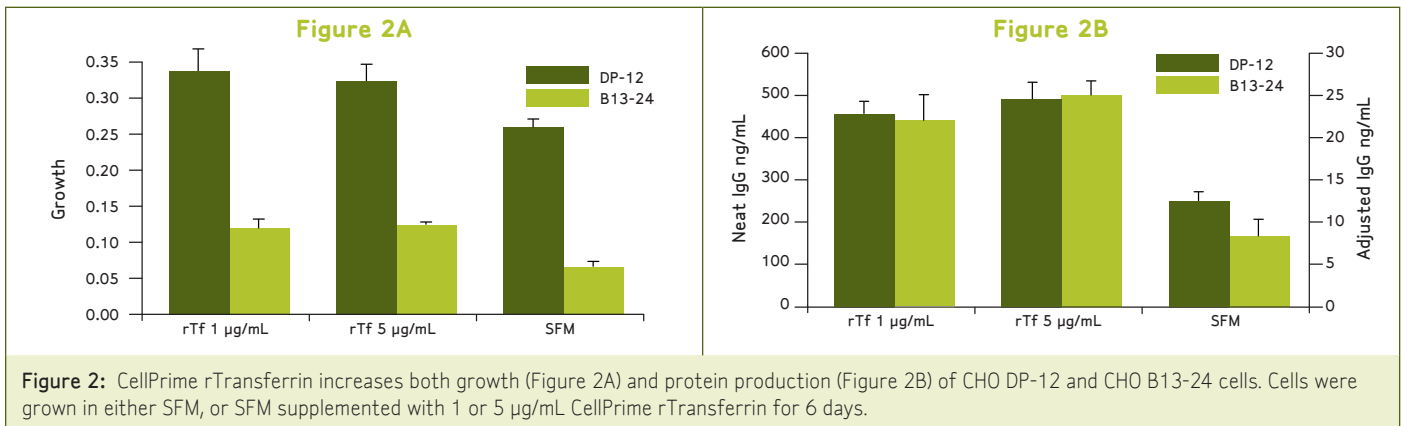
To examine the time-course of the effect of CellPrime rTransferrin on CHO cell growth and protein production, two concentrations of the supplement (1 and 5 µg/mL) were initially tested in CHO DP-12 cells. At day 6, modest increases in both

growth (Figure 1A) and protein production (Figure 1B) were observed as compared to the SFM control. By day 10, however, both concentrations of CellPrime rTransferrin had increased growth by >2-fold (Figure 1A) and increased protein production by >3-fold as compared to the SFM control (Figure 1B).



The ability of CellPrime rTransferrin to promote cell growth was also tested against DP-12 and B13-24 cell lines in parallel. At harvest (Day 6), both cell lines showed an increase in growth (Figure 2A) and protein production (Figure 2B) when compared to SFM. The increase in growth for the B13-24

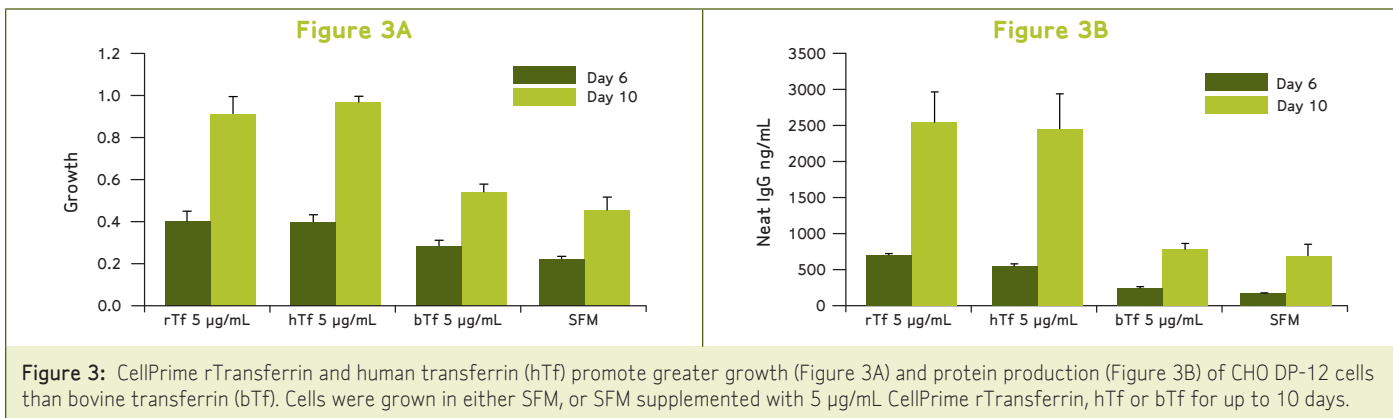
was modest compared to the increase demonstrated by the DP-12 cells; however, it was still a noteworthy increase over the SFM control. More importantly, the protein production of both cell lines increased by greater than 2-fold compared to SFM alone at both 1 and 5 µg/mL CellPrime rTransferrin.



Human Recombinant and Native Transferrins Promote Greater Growth and Protein Production than Bovine Transferrin

The next step was to compare the efficacy of CellPrime rTransferrin to commonly used transferrin media supplements. To this end, CellPrime rTransferrin was compared to serum-derived human transferrin (hTf) and bovine transferrin (bTf) at identical concentrations (5 µg/mL) in the CHO DP-12 cell line.

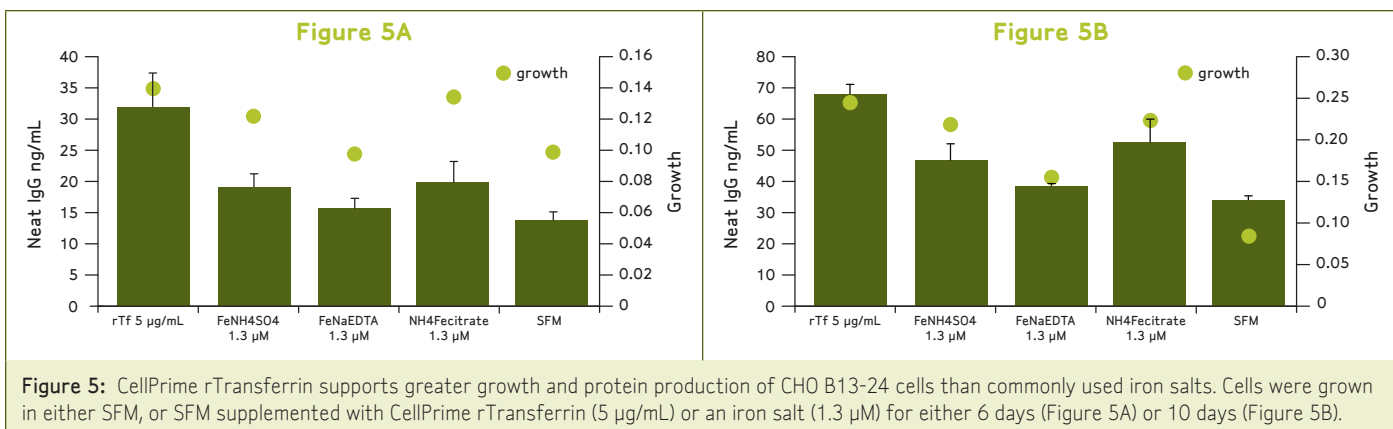
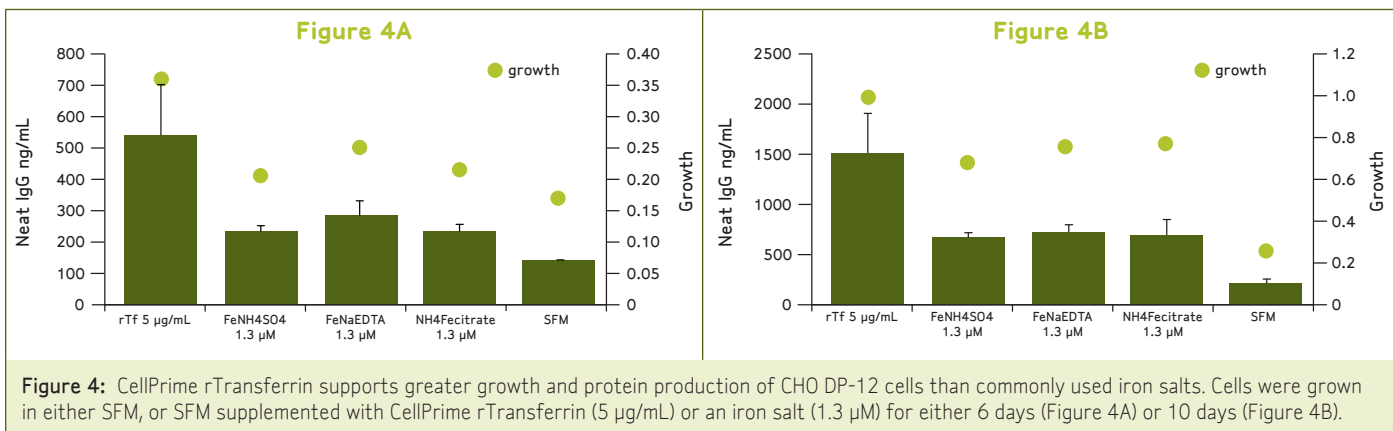
At day 6 and 10 in culture, CellPrime rTransferrin promoted similar increases in growth (Figure 3A) and protein production (Figure 3B) as native hTf. In contrast, bTf showed little increase in cell growth as compared to SFM, with significantly greater effects observed at day 10 compared to day 6. Similar results were observed at day 10 in culture (data not shown). This data suggests that CHO cells respond preferentially to human transferrin in either native or recombinant form.



CellPrime rTransferrin is More Effective at Promoting Growth and Protein Production than Iron Salts

Iron salt supplementation is another commonly used method for enhancing CHO cell performance. In this experiment, iron salts were added to a commonly used off-the-shelf medium (DMEM/F12) that already contains a nominal amount of iron. For these experiments, CellPrime rTransferrin (5 µg/ml) was tested against iron salts (1.3 µM) using the same experimental model as described above in CHO DP-12 (Figure 4A and 4B) and CHO B13-24 (Figure 5A and 5B) cells. The results indicate that all treatments with CellPrime rTransferrin performed

equal to or better than, treatments with iron salts for both growth and protein production in either CHO clone. The increased growth and protein production were again more pronounced in the DP-12 cells than the B13-24 cells, as observed in experiments described above. These data indicate that CellPrime rTransferrin’s benefit is derived from its biological activity as an iron carrier rather than from the addition of iron bound to CellPrime rTransferrin, since the amount of iron added via the CellPrime rTransferrin is much lower than that added to the medium by adding iron salts.



DISCUSSION

The biopharmaceutical industry is constantly striving for optimal cell growth and protein production in their upstream manufacturing process, while maintaining product/process consistency and compliance. Historically, the number of animal free, defined protein supplements available for use as industrial cell culture supplements has been limited. The introduction of CellPrime rTransferrin provides an animal-free alternative to iron salt supplementation that permits biological, not chemical, iron supplementation in cell culture media. This is an important distinction, as the data comparing the effect of CellPrime rTransferrin to iron salts suggest that biological delivery of iron promotes growth and protein production to a degree not seen with iron salts alone. The data detailed in this paper also demonstrate that CellPrime rTransferrin supports cell growth and protein

production equivalent to hTf, and superior to bTf, indicating that supplement species choice can substantially affect cell culture performance as well. The low concentration of CellPrime rTransferrin required to achieve increased cell performance makes this a cost-effective option for providing optimal iron supplementation to cells.

In summary, CellPrime rTransferrin provides a superior alternative to commonly used bovine-derived transferrin or iron supplements in cell culture by eliminating the use of animal-derived components while enhancing cell performance. Additional experiments are planned in customer-relevant assays on a larger scale (i.e., shake flask and bioreactor scale) in cells grown in completely serum-free conditions to verify the findings of this study.

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