

## **PROXYFERRIN supplement for transferrin-independent iron transport**

Information from Biochrom AG

PROXYFERRIN is a chemically defined low molecular substitute for holo-transferrin supporting the transferrin-independent iron transport into in vitro cultured mammalian cells. PROXYFERRIN's proprietary composition is free of animal derived components, pyrogens and proteins. The concentrated substitute combines several benefits as negligible biohazard, low costs compared to recombinant transferrin, suitability for many cell types, particularly low iron tolerant cells, endotoxin content below detection limit and stability upon storage. The main application of PROXYFERRIN offers versatility and safety for the design of hormonally defined serum-free cell culture media. Additionally it can provide improved cell proliferation stimulating activity to low serum supplemented media, as well as to the activity of serum supplements with low iron status.

### **1 Iron-donor in serum-free media is transferrin**

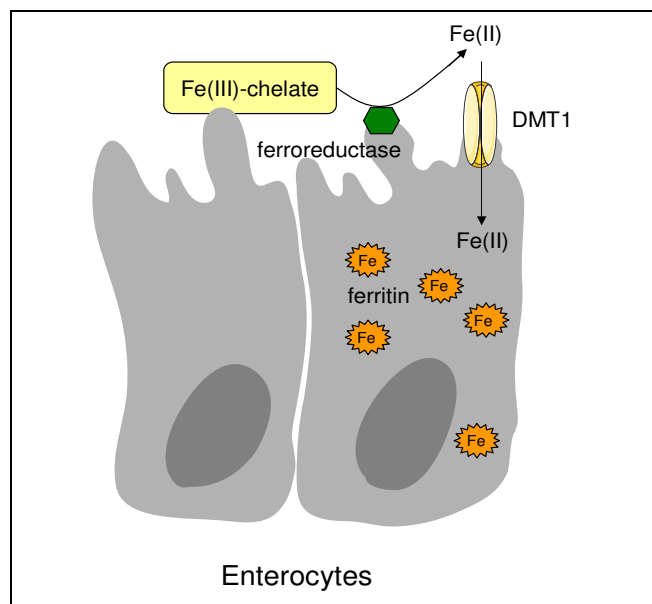
Ionised iron ( $\text{Fe}^{3+}/\text{Fe}^{2+}$ ) is essential for the mammalian metabolism because of the ease with which it can change its oxidative state. This makes iron a suitable component of hemoglobin, myoglobin, cytochromes and various non-heme enzymes. Iron is essential for all eukaryotes but can also be toxic; the same property that allows iron to gain and lose electrons can donate electrons to oxygen, resulting in the generation of superoxide anion radical and the hydroxyl radical via Fenton and Haber-Weiss reactions. An excess of reactive oxygen species readily converts biological molecules, including proteins, lipids and DNA thereby destroying their biochemical function. Consequently, all mammalian species tightly regulate the concentration of iron in biological fluids. For this reason iron supplements are essentials in the formulation of mammalian cell culture media. Most frequently applied as the iron-donor in serum-free media is **transferrin**. This plasma protein binds  $\text{Fe}^{3+}$  with high affinity. At neutral pH in plasma, transferrin- $\text{Fe}^{3+}$  binds to the transferrin receptor on the cell surface from where it is internalized by receptor-mediated endocytosis.

Alternatively *in vivo* and *in vitro* the **uptake of non-transferrin bound Fe** has been studied extensively in enterocytes<sup>1</sup>, as well as in a wide variety of other mammalian cells (Table 1). Though the mechanism of transferrin-independent iron-transport is less well analyzed, some common features can be found in all cell types (Fig.1).

**Tab. 1: Some mammalian cell types studied with focus on transferrin-independent iron uptake.**

Mammalian cell type	Tissue origin	Species
Enterocytes	Intestine epithelium	Human
HeLa	Cervix carcinoma	Human
Hepatocytes	Liver	Rat
K562	Myelogeneous erythroleukemia	Human
Fibroblasts	Skin	Human
Vero	Kidney epithelium	Green monkey
SK-Hep1	Liver carcinoma	Human
Wish	Amnion cells	Human
BHK21	Kidney cells	Hamster
HepG2	Liver carcinoma	Human
CHO-K1	Ovary cells	Chinese hamster

**Fig.1: Schematic uptake of non-transferrin bound ferric ions in enterocytes.**



Beside natural ferric chelators like citrate, a number of synthetic types of siderophores have been tested in serum-free cell culture; ethylenediaminetetraacetic acid (EDTA), iminodiacetic acid (IDA), aurointricarboxylic acid and tropolone being the most frequently investigated.

## 2 PROXYFERRIN a transferrin replacement concentrate

This animal-component-free proprietary product of Biochrom AG was designed to replace the function of holo-transferrin allowing the alternative transferrin-independent iron uptake in a broad range of mammalian cell culture systems. PROXYFERRIN consists of two types of che-

late iron complexes. The major  $\text{Fe}^{3+}$  complex is provided by the natural bidentate citric acid complex. It prevents free ferric ions from precipitating as insoluble  $\text{FeO.OH.nH}_2\text{O}$ . The minor complex is a tetradentate synthetic chelate-compound. Additionally PROXYFERRIN potentiates the non-transferrin-bound iron uptake into the cell by means of chelated  $\text{Zn}^{2+}$ -ions, a property which recently was ascribed to specific zinc transporters<sup>3</sup> in the plasma membrane of mammalian cells.

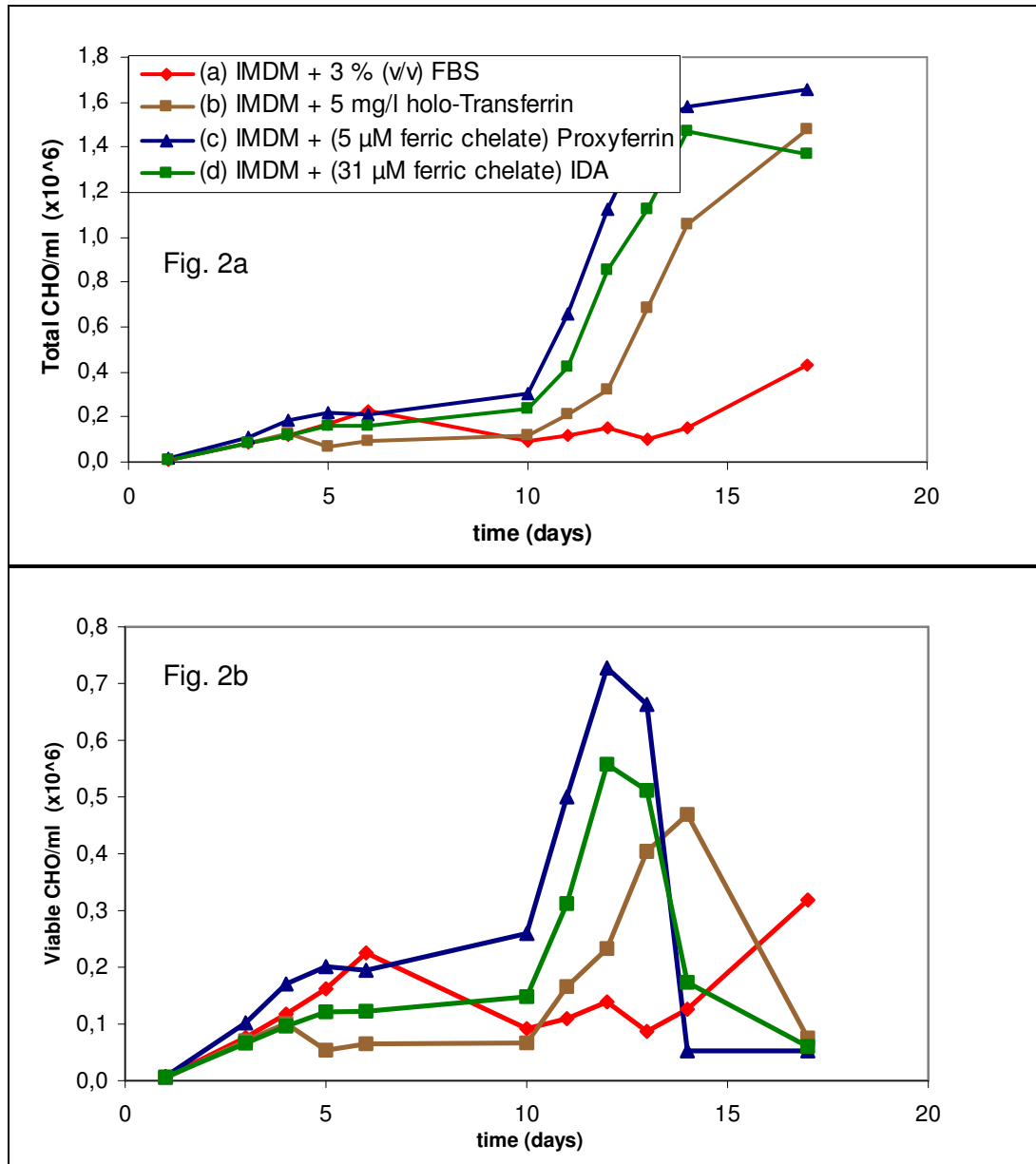
Whenever development of cell culture medium has to follow the safety requirements for therapeutic applications a replacement of native transferrin is necessary. Beside the lower biological risk of a protein-free substitute like PROXYFERRIN, a further advantage is given by the absence of contaminating bacterial endotoxin contaminations, which can accidentally be transferred into the culture medium when bound to added transferrin<sup>4</sup>. Therefore the use of a non-protein transferrin substitute facilitates the design of defined culture media by avoiding bio-hazards of animal components and by lower costs of the media components through substitution for recombinant transferrins.

The supplement concentration for transferrin-independent iron uptake should be adjusted to every cell type. For the majority of cells 1 ml of PROXYFERRIN-concentrate (Cat. No K370-02) per litre medium providing approx. 5  $\mu\text{M}$  ferric chelate is sufficient (low iron tolerant cells). However iron-consuming cells, e.g. the erythroid K562 cell line, where a high iron uptake is required for optimal heme synthesis, considerable larger (at least 20x) amounts of supplementation are needed (high iron dependent cells)<sup>5</sup>.

PROXYFERRIN has been tested in a number cell cultures grown in serum free defined media supplemented with various growth factor combinations.

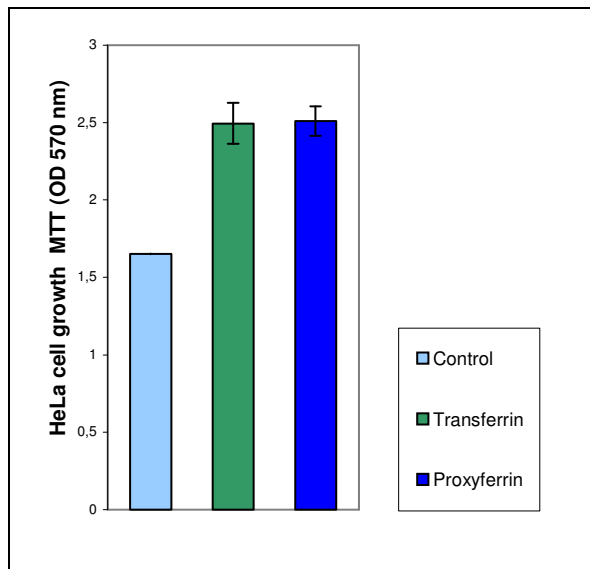
Fig.2a and 2b shows 17 days of continuous growth progression of Chinese hamster ovary (CHO) cells adapted to suspension and cultivated in defined medium supplemented with different ferric ion donor systems. After an adaption period of 10 days the viability of cells provided with an iron-uptake by transferrin-independent siderophores is maximal during exponential cell growth. Fig.2 clearly demonstrates, that under these culture conditions CHO proliferation in PROXYFERRIN supplemented media is by far superior as in FBS- or transferrin supplemented control cultures.

**Fig. 2a:** Suspension CHO batch cultures Iscove's modified Dulbecco medium (IMDM) basal medium supplemented with either a) 3 % (v/v) FBS or a serum-free defined growth-factor combination supplemented with b) 5 mg/l human holo-transferrin, c) 5  $\mu$ M ferric chelate (Proxylferrin) or d) 31  $\mu$ M ferric chelate (IDA). The cell proliferation during 17 days of continuous suspension culture was determined by counting the number of total cells. This cell count can be compared with the number of viable cells (Fig 2b).



An example for adherent cell culture is shown in Fig. 3. HeLa cells were cultivated on uncoated 96 well plates for 4 days in serum-free defined media (IMDM + combination of growth factors) supplemented without holo-transferrin (control), with 5µg/ml holo-transferrin and with PROXYFERRIN (5µM Fe<sup>3+</sup>-chelate; 1 ml/litre medium).

**Fig. 3: Influence of transferrin and PROXYFERRIN on the growth of HeLa cells. MTT assay was performed by measuring the extinction at 570nm.**

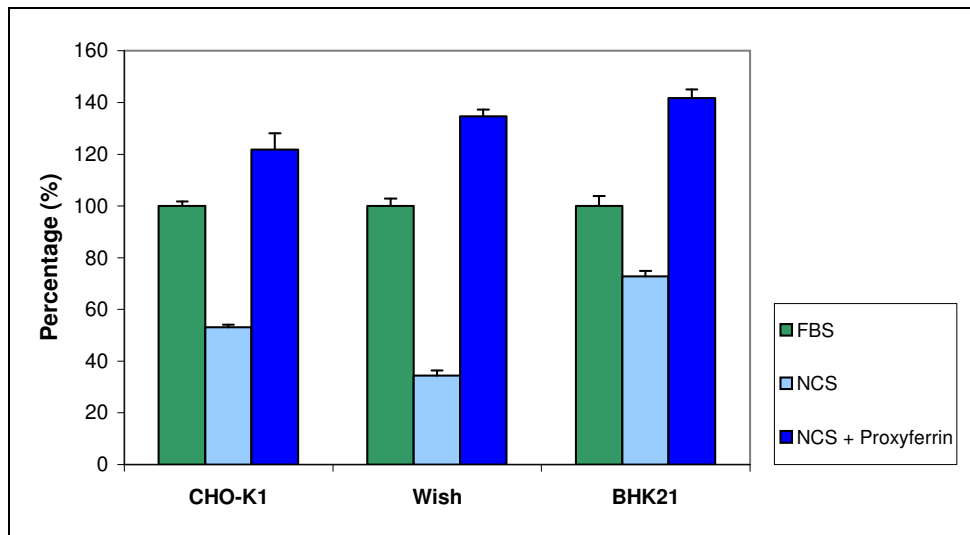


The influence of holo-transferrin on proliferation of HeLa cells can be fully restored, if the serum-free defined medium is supplemented with standard PROXYFERRIN concentration (1:1000 dilution of concentrate).

Thus, with respect to non-transferrin iron uptake, HeLa and CHO cells are typical representatives of low iron tolerant cells.

**Iron deficiency of newborn calf serum<sup>6</sup> (NCS)** can be compensated by means of PROXYFERRIN supplementation. Addition of PROXYFERRIN improves the proliferation capacity of NCS supplemented culture media. (Fig 4).

**Fig. 4: Proliferative response of 3 adherent mammalian cell lines cultivated 4 days in IMDM basal medium supplemented with 5% FBS, 5% NCS and 5% NCS + PROXYFERRIN. The extinction of MTT measurements for cell growth in FBS supplemented culture media was set to 100%. All cultures were performed in uncoated 96 well plates.**



## Literature

<sup>1</sup>M. Shindo et al. Functional role of DMT1 in transferrin-independent iron uptake by human hepatocyte and hepatocellular carcinoma cell, HLF.

*Hepatology Research*, Volume 35, Issue 3, Pages 152-162 (2006)

<sup>2</sup>J.M.Harrington & A.L.Crumbliss. The redox hypothesis in siderophore mediated iron uptake. *Biometals* 22:679-689 (2009)

<sup>3</sup>J.P. Liuzzi et al. Zip 14 (Slc39a14) mediates non-transferrin-bound iron uptake into cells. *PNAS*. 103: 37, 13612 -13617 (2006).

<sup>4</sup>D. Berger et al. Demonstration of an interaction between transferrin and lipopolysaccharide. An *in vitro* study. *European Surgical Research*. 23: 309-316 (1999).

<sup>5</sup>J. Kovar et al. The inability of cells to grow in low iron correlates with increasing activity of their iron regulatory protein (IRP). *In Vitro Cell. Dev. Biol Animal* 33:633-639 (1997)

<sup>6</sup>S-I.Kume & S.Tanabe Effect of Supplemental Lactoferrin with Ferrous Iron on Iron Status of Newborn Calves. *J. Dairy Sci*, 79:459-464 (1995).