Safe, Consistent Iron Delivery in Serum-Free Systems with Optiferrin® Recombinant Transferrin

Jacob Weber, PhD, and Mark Stathos, PhD
InVitria, Inc., USA

APPLICATION BENEFITS

- ***Optiferrin® (recombinant transferrin) delivers bioavailable iron via transferrin receptor-mediated uptake, mimicking the native iron delivery pathway.***
- ***After releasing iron inside the cell, transferrin is recycled back to the surface to repeat the cycle—supporting continuous, regulated iron uptake.***
- ***Enables robust cell proliferation in serum-free and chemically defined media, with performance equivalent to plasma-derived transferrin.***
- ***Animal-origin-free and blood-free, eliminating adventitious agent risk and simplifying regulatory compliance across biomanufacturing workflows.***

INVITRIA SOLUTIONS

Optiferrin - Recombinant human transferrin



EXECUTIVE SUMMARY

Efficient iron delivery is essential for cell growth and viability in vitro. Transferrin is widely used in cell culture media to fulfill this role. However, serum-derived transferrin poses challenges including batch variability, pathogen transmission risk, and regulatory complexity. InVitria's Optiferrin is a recombinant human transferrin produced in a non-mammalian expression system that overcomes these limitations.

In this Application note, we show that Optiferrin performs equivalently to plasma-derived transferrin in iron delivery and hybridoma cell proliferation, validating its use in serum-free, animal-origin-free work-flows.

Key Highlights

- Enables safe, receptor-mediated iron delivery
- Matches or exceeds performance of plasma-derived transferrin
- Supports proliferation in multiple cell types
- Facilitates serum-free, animal-origin-free, GMP-ready workflows

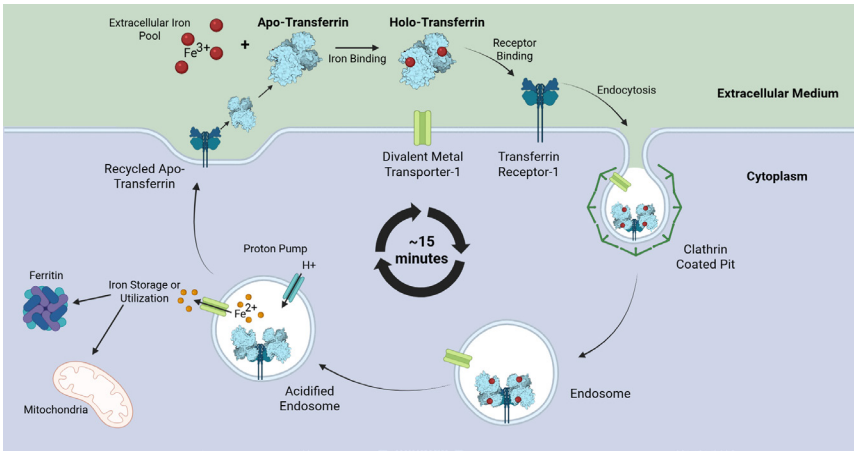


Figure 1. *Transferrin delivers iron through a tightly regulated endocytic cycle, enabling efficient, serum-free cellular uptake in just ~15 minutes allowing the iron-depleted transferrin (apo-transferrin) to be released from the cell to pick up additional iron to repeat the cycle.*

INTRODUCTION

Iron uptake is fundamental to cell metabolism, proliferation, and survival (Crescenzi et al., 2023). In living organisms, transferrin is the principal iron-binding protein in blood plasma. Human serum transferrin is a bilobal ~75 kD glycoprotein produced in the liver and found in blood plasma at approximately 2.5 mg/mL as a heterogeneous population: diferric (holo), monoferric N-lobe, monoferric C-lobe, or apo transferrin (Steere et al., 2012; Luck & Mason, 2012). Transferrin tightly binds Fe^{3+} ions and delivers them to cells via interaction with transferrin receptors (TFR) on the cell surface. Apo-transferrin has an extremely high affinity for ferric iron, with binding constants $K'_1 = 4.7 \times 10^{20}\ M^{-1}$ and $K'_2 = 2.4 \times 10^{19}\ M^{-1}$. This ensures that apo-transferrin rapidly binds available iron in serum or culture media, quickly converting to holo-transferrin (Aisen et al., 1978). This receptor-mediated endocytosis ensures iron is supplied in a controlled, bioavailable form, supporting cellular processes while minimizing oxidative stress and toxicity from free iron (Luck & Mason, 2012; Lane et al., 2015).

In cell culture, transferrin is widely used as a supplement in serum-free and chemically defined media to mimic its physiological role in iron delivery (Luck & Mason, 2012). Historically, transferrin has been purified from human serum; however, this approach carries risks related to batch variability, pathogen transmission, and regulatory hurdles (Steere et al., 2012).

To address these limitations, recombinant human transferrin products such as Optiferrin have been developed using a non-mammalian expression system. Recombinant transferrin is structurally and functionally equivalent to plasma-derived transferrin, offering the same reversible iron binding, transferrin-receptor binding, and ability to promote cell proliferation and productivity.

RESULTS AND DISCUSSION

Optiferrin Delivers Bioavailable Iron via Receptor-Mediated Endocytosis

At physiological pH, transferrin binds Fe^{3+} and interacts with transferrin receptor 1 (TFR1) on the cell surface, initiating endocytosis and iron release within the endosome (Lane et al., 2015). This receptor-mediated process ensures controlled iron uptake and prevents oxidative stress from free iron in the medium (Kawabata, 2022).

Holo transferrin (diferric transferrin) exhibits the highest affinity for the cell surface transferrin receptor (TFR1), followed by the two monoferric forms (FeNhTF and FeChTF) (Luck & Mason, 2012). One of these three iron-bound forms of transferrin will bind the TFR1 and be internalized via clathrin-dependent endocytosis. The internalization of transferrin is rapid, with a half-life of approximately 3.5 minutes at 37°C (Steere et al., 2011). Subsequent acidification of the endosome through ATP-dependent H+ pumps triggers the receptor-stimulated release of iron from transferrin, where Fe^{3+} is reduced to Fe^{2+} before export to the cytosol (Luck & Mason, 2012).

The now iron-free transferrin/TFR1 complex is subsequently redirected back to the cell surface, where the weak association of apo transferrin for the TFR1 at serum pH leads to its dissociation, allowing apo transferrin to bind additional Fe^{3+} and repeat the cycle. Each full transferrin receptor cycle—from ligand binding to iron release and recycling—is completed in roughly 15–16 minutes, with about 90% of transferrin recycled within 30 minutes. A smaller portion follows slower recycling pathways, requiring up to 2 hours. These kinetics vary depending on cell type and conditions (Steere et al., 2011).

Although the fully saturated diferric transferrin has the highest affinity for the TFR (Kd ~4 nM), the fact that monoferric forms also are capable of high-affinity bonds with the TFR (Kd ~36 and 32 nM for the FeNhTF and FeChTF, respectively) would indicate that partially iron-saturated transferrin would be functional in cell culture (Luck & Mason, 2012).

These tightly regulated and rapid kinetics make transferrin a preferred and physiologically relevant source of iron in chemically defined media. Optiferrin has been shown to fully replicate this iron delivery mechanism. Its ability to bind TFR1 and participate in iron transport demonstrates functional parity with native, serum-derived transferrin.

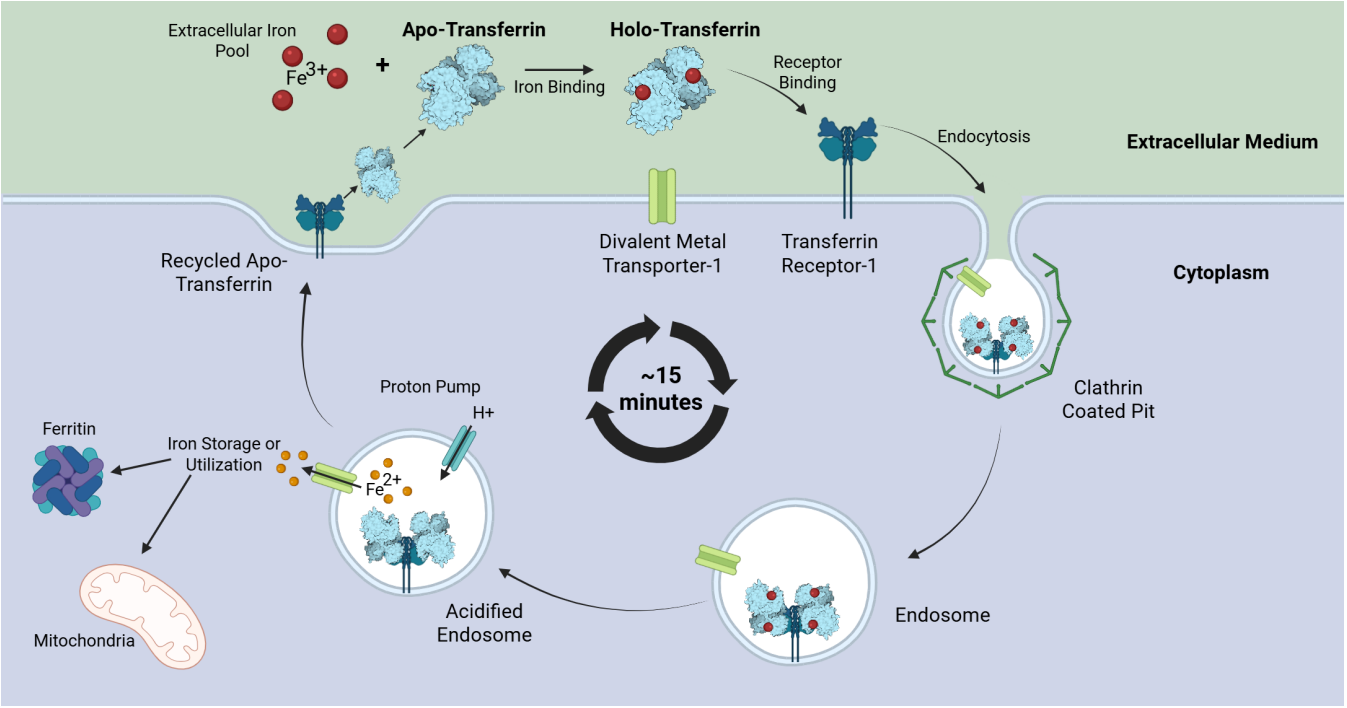


Figure 1: *Iron delivery and recycling of transferrin. Apo-Transferrin in the extracellular space readily binds to two ferric iron (Fe3+) ions. Iron-loaded transferrin (holo-transferrin) binds to transferrin receptor-1 on the cell surface and is internalized via clathrin-mediated endocytosis. Acidification via a proton pump within the endosome triggers iron release from transferrin. Released iron is reduced to its ferrous form (Fe2+) and is transported out of the endosome by divalent metal transporter-1. The now apo-transferrin and its receptor are recycled back to the cell surface where they dissociate and repeat the cycle again. This tightly regulated mechanism occurs over the span of ~15 minutes and enables safe, efficient iron delivery in serum-free media.*

Recombinant Optiferrin Retains Native Structure and Receptor Binding

Optiferrin is expressed at high levels in a non-mammalian expression system and has been rigorously analyzed to confirm the biochemical and functional aspects of this recombinant transferrin (Steere et al., 2012). Structural analysis verified that Optiferrin possesses the correct sequence and size and was able to compete with human serum-derived transferrin for TFR binding sites on both CCL-2 and Caco-2 cells (Zhang et al., 2012).

Optiferrin Supports Hybridoma Growth Comparable to Plasma-Derived Transferrin

Optiferrin demonstrated equivalent biological activity to native transferrin in supporting hybridoma cell proliferation across a relevant concentration range (0.1–10 mg/L). After 72 hours of culture in chemically defined conditions, viable cell counts were comparable between groups supplemented with Optiferrin and those with serum-derived transferrin.

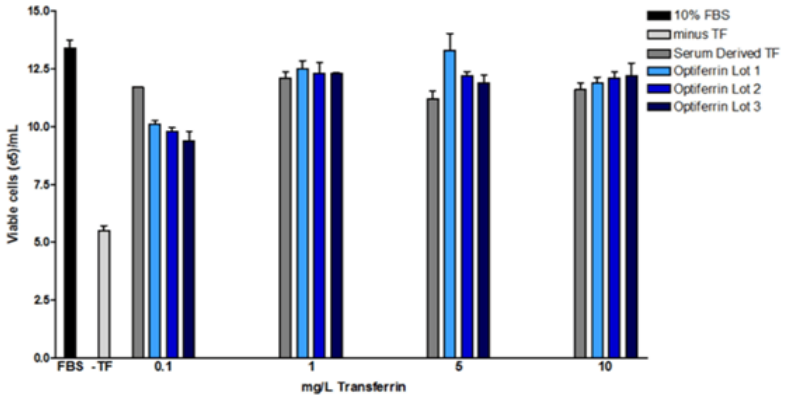


Figure 2: *Optiferrin supports hybridoma proliferation equivalent to serum-derived transferrin.Sp2/0 hybridoma cells cultured in serum-free media with Optiferrin (0.1–10 mg/L) exhibited cell viability comparable to those supplemented with human Plasma-derived transferrin. Data demonstrate Optiferrin's functional equivalence and suitability for serum-free workflows.*

Optiferrin Supports Stem Cell Differentiation and Maturation Through Efficient Iron Recycling and Delivery

Additionally, Dr. Paul Burridge's laboratory at Northwestern University has employed Optiferrin in chemically defined media to support the differentiation and maturation of human induced pluripotent stem cells (hiPSCs), including cardiomyocytes. Their studies demonstrate that Optiferrin enables consistent iron delivery in xeno-free systems without compromising functional, metabolic, or transcriptional fidelity of derived cell types (Fetterman et al., 2024). Further, Burridge and colleagues have used Optiferrin in multiple iPSC-based models of cardiovascular disease and drug testing, underscoring its compatibility with high-performance, translational cell systems (Burridge et al., 2016; Sharma et al., 2017).

MATERIALS & METHODS

Sp2/0 hybridoma cells (ATCC) were cultured in DMEM/F12 medium supplemented with GlutaMax, 10 mM HEPES, and 10% fetal bovine serum (FBS) under standard conditions. For transferrin bioactivity assays, cells were washed thoroughly with basal DMEM/F12 to remove residual serum and then seeded into fresh basal medium containing the following supplements:

- 1 g/L recombinant human albumin (rHSA)
- 10 mg/L recombinant human insulin
- 6.7 µg/L sodium selenite
- 2 mg/L ethanolamine

Cells were then treated with either serum-derived transferrin or recombinant human transferrin (Optiferrin) at concentrations ranging from 0.1 to 10 mg/L. Cultures were incubated for 72 hours, after which viable cell counts were assessed to evaluate transferrin-dependent proliferation.

CONCLUSION

Optiferrin is a fully animal-origin-free, recombinant transferrin, making it ideally suited for workflows requiring stringent regulatory compliance and the elimination of adventitious agent risk. Its structural and functional equivalence to native human transferrin has been validated across a wide range of cell types—including hybridomas, mesenchymal stem cells, neural stem cells, induced pluripotent stem cells, T cells, fibroblasts, keratinocytes, VERO cells, and hematopoietic stem cells. This broad compatibility makes Optiferrin a highly versatile supplement for serum-free and chemically defined media.

As an animal-origin-free iron carrier, Optiferrin
offers a consistent and scalable solution that supports robust cell proliferation, simplifies regulatory approval, and enables the transition to fully defined, xeno-free systems critical to modern biomanufacturing and cell therapy development.

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ORDERING INFORMATION

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Optiferrin - Recombinant Human Transferrin, 1 g vial	777TRF029-1g
Optiferrin - Recombinant Human Transferrin, 10 g bottle	777TRF029-10g

ABOUT INVITRIA

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InVitria, Inc.
2718 Industrial Drive
Junction City, KS 66441 USA
Phone: 800-916-8311
Fax: 785-238-5688
Email: info@invitria.com
www.invitria.com