

apo-Transferrin human Cell Culture Tested

Product Number **T1147**

Storage Temperature 2-8 °C

Product Description

CAS Number: 11096-37-0

Molecular Weight: approximately 76-81 kDa

λ_{\max} : 280 nm¹

Extinction Coefficient: $E^{\text{mM}} = 11.2$ (water)

This product is supplied as a lyophilized powder of human apo-transferrin and has been tested in cell culture (1 mg/ml). It is appropriate for use in cell culture applications.

Transferrin is a glycoprotein with homologous N-terminal and C-terminal iron-binding domains.² Transferrin is related to several other iron-binding proteins including lactoferrin, melanotransferrin, and ovotransferrin. These molecules comprise the transferrin superfamily. All members of this superfamily have similar polypeptide folding patterns. The N-terminal and C-terminal domains of these proteins are globular moieties of about 330 amino acids. Each of these domains is divided into two sub-domains, with the iron- and anion-binding sites found within the intersubdomain cleft. The binding cleft opens with iron release, and closes with iron binding. Transferrin binds iron with an association constant of approximately 10^{22} M^{-1} .³ Ferric iron couples to transferrin only in the presence of an anion (usually carbonate) that serves as a bridging ligand between metal and protein, excluding water from the two coordination sites.³⁻⁵ Without the anion cofactor, iron binding to transferrin is negligible. In the presence of anions, ferric transferrin is resistant to all but the most potent chelators. The remaining four coordination sites are provided by the transferrin protein - a histidine nitrogen, an aspartic acid carboxylate oxygen, and two tyrosine phenolate oxygens.^{6,7} Available evidence suggests that anion-binding takes place prior to iron-binding. Iron release from transferrin involves protonation of the carbonate anion, loosening the metal-protein bond.

The sum of all iron binding sites on transferrin constitutes the total iron binding capacity (TIBC) of plasma. Under normal circumstances, approximately one-third of transferrin iron-binding pockets are filled. Consequently, with the exception of iron overload where all the transferrin binding sites are occupied, non-transferrin-bound iron in the circulation is virtually nonexistent. The normal half-life of iron in the circulation is about 75 minutes.⁸ The absolute amount of iron released from transferrin per unit time is the plasma iron turnover (PIT). Radioactive tracer studies indicate that at least 80% of the iron bound to circulating transferrin is delivered to the bone marrow and incorporated into newly formed erythrocytes.^{9,10} Other major sites of iron delivery include the liver, which is a primary depot for stored iron, and the spleen. Hepatic iron is found in both reticuloendothelial cells and hepatocytes.¹¹

Iron is taken into cells by receptor-mediated endocytosis of monoferric and diferric transferrin.¹²⁻¹⁴ Receptors on the outer face of the plasma membrane bind iron-loaded transferrin with a very high affinity. The C-terminal domain of transferrin appears to mediate receptor binding.¹⁵ Diferric transferrin binds with higher affinity than monoferric transferrin or apotransferrin.^{16,17} The dissociation constant (K_d) for bound diferric transferrin ranges from 10^{-7} M to 10^{-9} M at physiologic pH, depending on the species and tissue assayed.^{18,19} The K_d of monoferric transferrin is approximately 10^{-6} M . The concentration of circulating transferrin is about 25 mM. Therefore, cellular transferrin receptors ordinarily are fully saturated.

After binding to its receptor on the cell surface, transferrin is rapidly internalized by invagination of clathrin-coated pits with formation of endocytic vesicles.^{20,21} Following internalization into endosomes, the transferrin-receptor complex is subjected to a drop in endosomal pH (pH lowered to 5.5),^{22,23} which weakens the association between iron

and the transferrin. With the assistance of an oxidoreductase, the bound iron is then reduced from the Fe³⁺ state to Fe²⁺, leading to the release of the iron from the transferrin.^{24,25} Conformational changes in the transferrin receptor also play a role in this iron release.^{26,27}

Following the release of iron, receptor-bound apo-transferrin recycles to the cell surface rather than being transported to lysosomes for degradation. The neutral pH at the cell surface promotes the release of the apo-transferrin from its receptor,¹⁵ where it can again circulate and bind additional iron to undergo further rounds of iron delivery to cells.^{13,22,23} The average transferrin molecule, with a half-life of eight days, may be used up to one hundred times for iron delivery.²⁸

To form holo-transferrin (i.e., to saturate apo-transferrin with iron), the following procedure can be followed. The apo-transferrin is mixed with 2% of its mass in ferrous ammonium sulfate hexahydrate (Product No. F 3754) with sodium carbonate buffer, pH 5.9, for 1.5 hours. The pH is then raised to 8.5 with sodium carbonate and the solution is mixed for an additional 1.5 - 2 hours. The sample is then dialyzed against water to remove the buffer salts.

Precautions and Disclaimer

For Laboratory Use Only. Not for drug, household or other uses.

Preparation Instructions

This product is soluble in water (2 mg/ml).

Storage/Stability

Aqueous solutions of apo-transferrin should be stable at 4 °C for 5-10 days. Solutions should be filter-sterilized for maximum stability.

References

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