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Datasheet for 009-0334

Human Transferrin Peroxidase

Overview

Description:	Human Transferrin Peroxidase Conjugated - 009-0334
Item No.:	009-0334
Size:	1 mg
Applications:	Dot Blot, EM, IF
Origin:	Human

Product Details

Background:	Human transferrin is encoded by the TF gene and is an iron-binding blood plasma glycoprotein that controls the level of free iron in biological fluids. Human transferrin binds iron very tightly but reversibly. Human transferrin is the most important iron pool in mammals. Human transferrin has a molecular weight of around 80 kDa and contains 2 specific high-affinity Fe(III) binding sites. The affinity of Human transferrin for Fe(III) is extremely high but decreases progressively with decreasing pH below neutrality. Human Transferrin also plays a role in the immune system, creating environments low in iron for which many pathogenic bacteria are unable to thrive.
Synonyms:	Human transferrin peroxidase conjugation, HRP conjugated transferrin
Species of Origin:	Human
Conjugate:	Peroxidase (HRP)
Format:	Transferrin
Type:	Native Protein

Target Details

Gene Name:	TF
Purity/Specificity:	Human Transferrin Peroxidase conjugated was prepared from normal serum by a multi-step process including selective precipitation and tandem chromatography followed by extensive dialysis against the buffer stated above. Human Transferrin Peroxidase conjugated was assayed by immunoelectrophoresis and resulted in a single precipitin arc against anti-Peroxidase, anti-Human Transferrin and anti-Human Serum.

Relevant Links:	• GeneID - 7018 • NCBI - AAB22049.1 • UniProtKB - Q06AH7

Application Details

Tested Applications:	Dot Blot
Suggested Applications:	EM, IF (Based on references)
Application Note:	Human Transferrin Horseradish Peroxidase (HRP) has been tested in dot blot and is a suitable protein for use as a control reagent in both Western Blotting and ELISA experiments.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	4,000 - 10,000
IHC:	200 - 2,000
WB:	2,000 - 5,000

Formulation

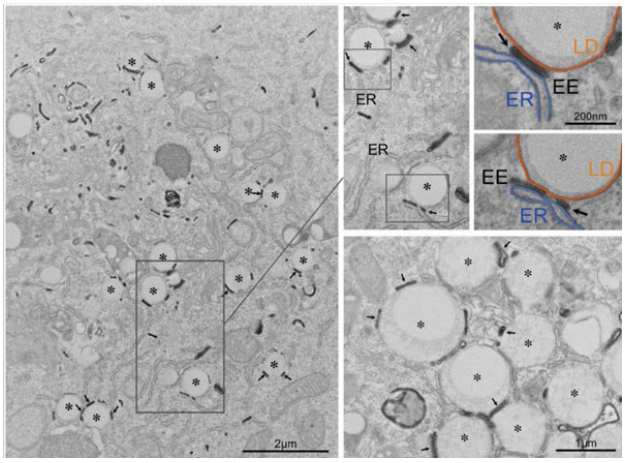
Physical State:	Lyophilized
Concentration:	1.0 mg/mL by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	0.01% (w/v) Gentamicin Sulfate. Do NOT add Sodium Azide!
Stabilizer:	10 mg/mL Bovine Serum Albumin (BSA) - Immunoglobulin and Protease free
Reconstitution Volume:	1.0 mL
Reconstitution Buffer:	Restore with deionized water (or equivalent)

Shipping & Handling

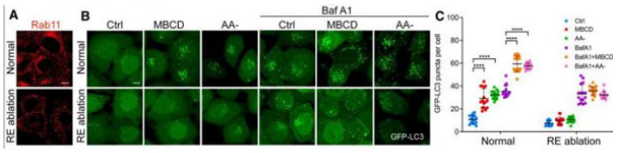
Shipping Condition:	Ambient
Storage Condition:	Store vial at 4° C prior to restoration. For extended storage aliquot contents and freeze at -20° C or below. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. Human Transferrin Peroxidase conjugated is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.

Expiration: Expiration date is one (1) year from date of receipt.

Images



Immunohistochemistry
The figure illustrates that EE tubules, defined by the presence of internalized transferrin, interact with LDs in mammalian cells. BHK cells were cotransfected with a plasmid encoding human transferrin receptor (TfR plus plasmid encoding GFP) and then incubated with transferrin-HRP for 30 min at 37°C. Cells were then processed for HRP detection and processed for electron microscopy using a mild fixation/low membrane-contrast staining method to optimize transferrin-HRP visualization. Transferrin-HRP-labeled EE tubules (arrows) were specifically associated with LDs (asterisks) as shown in the low magnification overview (left panel) and at higher magnification in the lower right panel. The two pseudocolored panels show higher magnification views of the neighboring panels, with ER in blue and the LD monolayer in orange; note the tripartite interaction with the EE tubule (EE, arrows). PMID: 32404335.



Immunofluorescence Microscopy
CEMM disruption releases VAMP3 from CEMMs at recycling endosomal membrane.
Used a recycling endosome ablation approach by using combined treatment with 3,3′-diaminobenzidine (DAB) and H2O2 to the cells pre-loaded with horseradish peroxidase-transferrin (HRP-TF).60,61 The significant reduction of the RAB11 signaling in the recycling endosome-ablated cells (RE ablation) proved the ablation efficiency of this method (Figure 3A). Importantly, CEMM disruption-induced autophagic flux is almost totally blocked by RE ablation (Figures 3B and 3C), indicating the importance of recycling endosomes in CEMM disruption-induced autophagosome formation
(A) After ablation of recycling endosomes, HeLa cells were immunostained with Rab11 (red), and observed under a confocal microscope (×600). Scale bars, 5 μm. (B) After ablation of recycling endosome, HeLa cells with stable expression of GFP-LC3B were pre-treated with MBCD (5 mM, 1 h) and then incubated in the presence or absence of Baf A1 (100 nM). Then cells were observed under a confocal microscope (×600). Scale bars, 5 μm. (C) The number of GFP-LC3 puncta observed in (B) are presented as means ± SD. ????p < 0.0001.
Figure 3. PMID: 34786475.

Dot Blot
Dot Blot result of Human Transferrin Peroxidase conjugate. Dots are Human Transferrin HRP at (1) 100ng, (2) 33.3ng, (3) 11.1ng, (4) 3.70ng, (5) 1.23ng. Blocking: MB-070 for 60 min at RT. Primary Antibody: none. Secondary Antibody: none. Imaged with BioRad ChemiDoc, Chemi filter.

References

- Shi Y et al. Cholesterol-enriched membrane micro-domain deficiency induces doxorubicin resistance via promoting autophagy in breast cancer. *Mol Ther Oncolytics.* (2021)
- Parton RG et al. Novel contact sites between lipid droplets, early endosomes, and the endoplasmic reticulum. *J Lipid Res.* (2020)

Disclaimer

No test method can provide total assurance that the hepatitis B virus, hepatitis C virus, human immunodeficiency virus, or any other infectious agents are absent. Thus, all blood products, including purified proteins derived from human blood sources, should be handled at Biosafety Level 2 as recommended by the CDC\NIH manual entitled Biosafety in Microbiological and Biomedical Laboratories for potentially infectious human serum, blood specimens or proteins derived from same. Source material for the human blood product supplied to your facility has been tested for the detection of HIV antibody, Hepatitis B surface antigen, antibody to Hepatitis C, HIV 1 antigen(s), antibody to HTLV - I/II, and syphilis by FDA guidelines. All units were found to be non-reactive/negative for these tests. All human blood source material is collected in FDA licensed centers and is tested with FDA approved test kits.

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