Datasheet for ABIN612787

Transferrin ELISA Kit

1 Publication

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Overview

Quantity:	96 tests
Target:	Transferrin (TF)
Reactivity:	Human
Method Type:	Competition ELISA
Minimum Detection Limit:	100 ng/mL
Application:	ELISA

Product Details

Purpose:	The AssayMax Human Transferrin ELISA (Enzyme-Linked Immunosorbent Assay) kit employs a quantitative competitive enzyme immunoassay technique that measures human plasma, and serum Transferrin
Brand:	AssayMax
Sample Type:	Plasma
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Components:	Human Transferrin Microplate: A 96 well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human Transferrin. Sealing Tapes: Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay. Human Transferrin Standard: Human Transferrin in a buffered protein base (60 µg, lyophilized). Biotinylated Transferrin: 1 vial, lyophilized. MIx Diluent Concentrate (10x): A 10-fold concentrated buffered protein base (30 ml). Wash Buffer Concentrate (20x): A 20-fold

Product Details

	concentrated buffered surfactant (30 ml). Streptavidin-Peroxidase Conjugate (SP Conjugate): A 100-fold concentrate (90µl). Chromogen Substrate: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml). Stop Solution: A 0.5 N hydrochloric acid to stop the chromogen substrate reaction (12 ml). 1
Material not included:	Microplate reader capable of measuring absorbance at 450 nm. Pipettes (1-20 µL, 20-200 µL, 200-1000µLand multiple channel). Deionized or distilled reagent grade water.

Target Details

Target:	Transferrin (TF)
Alternative Name:	Transferrin (TF Products)
Background:	Transferrin is a plasma protein that transports iron through the blood to the liver, spleen and bone marrow. Low transferring level in plasma could associate with anemia , and chronic liver disease. On the other hand, high plasma transferrin level could indicate iron deficiency anemia.
Pathways:	Transition Metal Ion Homeostasis

Application Details

Sample Volume:	25 µL
Assay Time:	< 2 h
Plate:	Pre-coated
Protocol:	A polyclonal antibody specific for human Transferrin has been pre- coated onto a 96-well microplate with removable strips. Transferrin in standards and samples is competed by a biotinylated Transferrin sandwiched by the immobilized antibody and streptavidin- peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.
Reagent Preparation:	Freshly dilute all reagents and bring all reagents to room temperature before use. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. MIx Diluent Concentrate (10x): Dilute the MIx Diluent 1:10 with reagent grade water. Store for up to 1 month at 2-8°C. Standard Curve: Reconstitute the 60 g of Transferrin Standard with 0.6 ml of MIx Diluent to generate a Stock Solution of 100 g/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare triplicate standard points by serially diluting the Stock Solution (100 g/ml) 1:4 with MIx Diluent to produce 25, 6.25, 1.56 and 0.39 g/ml solutions. MIx Diluent serves as the zero standard (0 g/ml). Any remaining solution should

	be frozen at -20°C. Standard Point Dilution [Transferrin] (g/ml) Stock Solution (100 g/ml) + 3 parts MIx Diluent P1 25.00 P2 1 part P1 + 3 parts MIx Diluent 6.25 P3 1 part P2 + 3 parts MIx Diluent 1.56 P4 1 part P3 + 3 parts MIx Diluent 0.39 P5 MIx Diluent 0.00 Biotinylated Transferrin (3x): Dilute Biotinylated Transferrin with 4 ml MIx Diluent to produce a 3-fold stock solution, which should be further diluted 1:3 with MIx Diluent. Any remaining solution should be frozen at -20°C. Wash Buffer Concentrate (20x): Dilute the Wash Buffer Concentrate 1:20 with reagent grade water. 2 SP Conjugate (100x): Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with MIx Diluent. Any remaining solution should be frozen at -20°C.
Sample Collection:	Plasma: Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 2000 x g for 10 minutes and assay. Dilute samples 1:2000 into MIx Diluent. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze- thaw cycles (EDTA or Heparin can also be used as anticoagulant). Serum: Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2000 x g for 10 minutes. Remove serum and assay. Dilute samples 1:2000 into MIx Diluent. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
Assay Procedure:	Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20 - 30 °C). Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator. Add 25 µL of standard or sample per well, and immediately add 25 µL of Biotinylated Transferrin to each well (on top of the Standard or sample) and mix gently. Cover wells with a sealing tape and incubate for one hour. Start the timer after the last sample addition. Wash five times with 200 µL of Wash Buffer. Invert the plate and decant the contents, and hit it 4-5 times on absorbent paper towel to complete remove liquid at each step. Add 50 µL of Streptavidin-Peroxidase Conjugate to each well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance. Wash five times with 200 µL of Wash Buffer. Add 50 µL of Chromogen Substrate per well and incubate for 10 minutes or till the optimal blue color density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip. Add 50 µL of Stop Solution to each well. The color will change from blue to yellow. Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. Please note that after the reaction is stopped for about 10 minutes, some black particles may be generated at high concentration point, which will reduce the readings.
Calculation of Results:	Calculate the mean value of the duplicate or triplicate readings for each standard and sample.

Application Details

	To generate a Standard Curve, plot 4-parameter graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit. Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor. Standard Curve The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.
Assay Precision:	Intra-assay and inter-assay coefficients of variation were 4.5% and 7.0% respectively.
Restrictions:	For Research Use only

Handling

Handling Advice:	The kit should not be used beyond the expiration date.
Storage:	4 °C/-20 °C
Storage Comment:	Store kit at 2-8°C or -20°C upon arrival up to the expiration date. Opened MIx Diluent may be stored for up to 1 month at 2-8°C. Store reconstituted reagents at -20°C or below. Opened unused strip wells may return to the foil pouch with the desiccant pack, reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator.

Publications

Product cited in:	Nikolic, Adams, Otahal, Edwards, Sharman: "Association of von Willebrand factor blood levels with exercise hypertension." in: **European journal of applied physiology**, Vol. 115, Issue 5, pp. 1057-65, (2015) (PubMed).