Datasheet for ABIN612772

Ceruloplasmin ELISA Kit

Go to Product page

Overview

Quantity:	96 tests
Target:	Ceruloplasmin (CP)
Reactivity:	Rat
Method Type:	Sandwich ELISA
Minimum Detection Limit:	300 pg/mL
Application:	ELISA

Product Details

Purpose:	The AssayMax Rat Ceruloplasmin ELISA kit is designed for detection of rat Ceruloplasmin in urine and cell culture supernatants
Brand:	AssayMax
Sample Type:	Cell Culture Supernatant
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Components:	Rat Ceruloplasmin Microplate: A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against rat Ceruloplasmin. Sealing Tapes: Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay. Rat Ceruloplasmin Standard: Rat Ceruloplasmin in a buffered protein base (40 ng, lyophilized). Biotinylated Ceruloplasmin Antibody (100x): A 100-fold biotinylated polyclonal antibody against rat Ceruloplasmin (80µl). 1 MIx Diluent Concentrate (10x): A 10-fold concentrated buffered protein base (30 ml). Wash Buffer Concentrate (20x): A 20-fold concentrated buffered

Product Details

	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).
Material not included:	Microplate reader capable of measuring absorbance at 450 nm. Pipettes (1-20 µL, 20-200 µL, 200-1000µLand multiple channel pipette) Deionized or distilled reagent grade water

Target Details

Target:	Ceruloplasmin (CP)
Alternative Name:	Ceruloplasmin (CP Products)
Background:	Ceruloplasmin is an abundant 2-serum glycoprotein that contains 95% of the copper found in the plasma of vertebrate species. Ceruloplasmin is a copper-binding protein that normally removes iron from cells by its ferroxidase activity. Ceruloplasmin concentration on average is 14.6 (4.0) mg/dl. Low levels of ceruloplasmin lead to the abnormal deposition of iron in cells, including those of the pancreas, liver, retina and the basal ganglia region of the brain. Some diseases associated with ceruloplasmin are Wilson's disease , Hemochromatosis , Menkes disease and Aceroluplasminemia.
Pathways:	Transition Metal Ion Homeostasis

Application Details

Sample Volume:	50 µL
Assay Time:	4 h
Plate:	Pre-coated
Protocol:	This assay employs a quantitative sandwich enzyme immunoassay technique that measures Ceruloplasmin in 4 hours. A polyclonal antibody specific for rat Ceruloplasmin has been pre-coated onto a microplate. Ceruloplasmin in standards and samples is sandwiched by the immobilized antibody and biotinylated polyclonal antibody specific for Ceruloplasmin, which is recognized by a 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. Ceruloplasmin Standard: Reconstitute the 40 ng of rat Ceruloplasmin Standard with 2.0 ml of MIx Diluent to generate a 20 ng/ml of solution. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare triplicate standard points by serially diluting the Standard solution (20 ng/ml) 1:2 with equal volume of MIx Diluent to produce 10, 5, 2.5, 1.25, 0.625 and 0.313 ng/ml. MIx Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C. Standard Point Dilution [Ceruloplasmin] (ng/ml) P1 1 part Standard (20 ng/ml) 20.00 P2 1 part P1 + 1 part MIx Diluent 10.00 P3 1 part P2 + 1 part MIx Diluent 5.00 P4 1 part P3 + 1 part MIx Diluent 2.50 P5 1 part P4 + 1 part MIx Diluent 1.25 P6 1 part P5 + 1 part MIx Diluent 0.63 P7 1 part P6 + 1 part MIx Diluent 0.31 P8 MIx Diluent 0.00 Biotinylated Ceruloplasmin Antibody (100x): Spin down the antibody briefly and dilute the desired amount of the antibody 1:100 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. 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:	Urine: Collect urine using sample pot. Centrifuge samples at 600 x g for 10 minutes and assay. Dilute samples 1:2 into MIx Diluent. Store samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles. Cell Culture Supernatants: Centrifuge cell culture media at 2000 x g for 10 minutes to remove debris. Collect supernatants and assay. The samples can be stored at -20°C or below. 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 50 µL of Standard or sample per well. Cover wells with a sealing tape and incubate for two hours. 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 Biotinylated Ceruloplasmin Antibody to each well and incubate for 1 hour. Wash five times with 200 µL of Wash Buffer as above. Add 50 µL of Streptavidin-Peroxidase Conjugate per 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 as above. Add 50 µL of Chromogen Substrate per well and incubate for about 10 minutes or till the optimal color density develops. Gently tap the plate to ensure thorough mixing and break the bubbles in the well with pipette tip. Add 50 µL of Stop

Application Details

	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 some unstable black particles may be generated at high optical densities to reduce the readings after stopping the reaction for about 10 minutes.
Calculation of Results:	Calculate the mean value of the duplicate or triplicate readings for each standard and sample. To generate a Standard Curve, plot the 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 5.0 % and 7.6% 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.