Ceruloplasmin Activity Colorimetric Assay Kit (#BN00893)

(Catalog # BN00893; 100 assays; Store at 4°C)

I. Introduction:

Ceruloplasmin is a copper containing protein found primarily in the blood. It carries approximately 70% of the total copper present in the blood. Ceruloplasmin exhibits a weak oxidase activity, which is a more accurate method of assessing ceruloplasmin in serum than immunodiffusion or other non-enzymatic assays. Normal Ceruloplasmin levels are generally 1-4 µM (15-60 mg/dl), equivalent to approximately 50-150 mU/ml. Elevated amounts of serum ceruloplasmin are found in pregnancy, cancer, rheumatoid arthritis and in several mental conditions such as Alzheimer's, schizophrenia and OCD. Abnormally low amounts are found in Wilson's and Menkes' Diseases and in several other rare conditions. Assay Genie's Ceruloplasmin Activity Assay Kit utilizes an oxidase substrate, which gives an intensely colored product upon oxidation. The kit is fast, easy to use and suitable for high throughput applications and can measure ceruloplasmin activity in serum in the range between approximately 5 and 500 mU/ml.

II. Application:

- Cancer research
- Neurodegenerative disease research
- Autoimmune disease research

III. Sample Type:

- Serum

IV. Kit Contents:

Components	BN00893	Cap Code	Part Number
Ceruloplasmin Assay Buffer	25 ml	WM	BN00893-1
Ammonium Sulfate, saturated (~4.1M)	10 ml	NM	BN00893-2
Ceruloplasmin Substrate	1 ml	Red	BN00893-3
Oxidizer (100 mM)	100 µl	Blue	BN00893-4
Stabilizer	100 µl	Orange	BN00893-5

V. User Supplied Reagents and Equipment:

- Microplate reader

VI. Storage and Handling:

Store kit at 4°C, protected from light. Briefly centrifuge small vials prior to opening. Since this is an enzyme assay, it should be temperature controlled. Set the plate reader to 25°C and place the Ceruloplasmin Assay Buffer into a water bath set at 25°C for 30 min. prior to use.

VII. Reagent Preparation and Storage Conditions:

All components are ready to use as supplied. Use within two months.

VIII. Ceruloplasmin Activity Assay Protocol:

1. **Sample Preparation:** Chloride in serum is an inhibitor of the ceruloplasmin enzyme activity and needs to be removed prior to analysis. This can be accomplished by Ammonium Sulfate precipitation of the serum proteins followed by removal of the supernatant containing the chloride or by dialysis of samples against a 1000X volume of dH_2O for 1 hour. For the Ammonium Sulfate method, take 100 µl of serum and add 100 µl of saturated Ammonium Sulfate, vortex briefly to mix then place on ice for 5 minutes. Centrifuge at 10k rpm at ambient T° for 5 min. to pellet the protein precipitate and remove 160 µl of the clear supernatant with a pipette. Add 160 µl of dH_2O to the pellet and dissolve. Add samples equivalent to 5-20 µl into 96-well plate and bring volumes to 100 µl with Ceruloplasmin Assay Buffer.
2. **Standard Curve:** Dilute the Oxidizer to 5 mM by adding 10 µl of 100 mM Oxidizer to 180 µl of Ceruloplasmin Assay Buffer. Add 10 µl Stabilizer and mix well. Add 0, 2, 4, 6, 8 & 10 µl of 5 mM Oxidizer into a series of wells in 96-well plate to generate 0, 10, 20, 30, 40 & 50 nmol Standard and bring the volume to 100 µl with Ceruloplasmin Assay Buffer.

   Note: The Standard Curve is produced by a nonenzymatic oxidation using chemical Oxidizer. The Stabilizer protects the color of the product for up to 15 min. so the Standard Curve has to be read within the same time (compatible timeframe for measuring the enzyme activity as well).
3. **Reaction Mix:** For each sample and Standard well, prepare 100 µl of Reaction Mix:

	Reaction Mix
Ceruloplasmin Substrate:	10 µl
Ceruloplasmin Assay Buffer:	90 µl

   Mix. Add 100 µl of the Reaction Mix to each sample and Standard well.
4. **Measurement:** Read OD in kinetic mode at 560 nm. The reaction is linear for only around 15-20 minutes and tends to appear to slow slightly after that. Cold samples (not adequately equilibrated to 25°C) will cause a slight lag phase detectable for the first 1-2 minutes. The Standard curve can read in endpoint mode (i.e., at the end of incubation time).
   Note: Ceruloplasmin does a 1 electron oxidation of the substrate to a red product. This product is increasingly unstable as its concentration increases and 2 molecules of the product undergo disproportionation to 1 molecule of substrate and 1 molecule of a 2 electron oxidation product which has a slightly lower absorbance than the 1 electron product at 560 nm. The enzymatic oxidation is linear over a wide range but the disproportionation results in a slight downward bend of the enzymatic reaction after around 15 minutes as the OD gets near and above 1.

5. Calculation: Plot the Standard Curve (OD vs. nmol) and determine the slope of the Standard Curve. For more accurate work, only use OD's less than 1 to determine the slope. Slope = OD/nmol of oxidized substrate.

Determine the linear portion of the curve for the samples and calculate the ΔOD/minute for this portion of the curve (equal to OD_2-OD_1/T_2-T_1), where OD_2 and OD_1 is absorbance at the end and beginning of linear portion, respectively and T_2 and T_1 is the time at the end and beginning of linear portion in minutes.

Sample Ceruloplasmin Activity = S_k/S_s/ V x 2 (mU/ml or U/L)

Where: **S_k** = kinetic slope of the sample (OD/min) in the linear portion of the curve

S_s = slope of the standard curve (OD/nmol)

V = volume of the sample in ml

2 = dilution factor (only for ammonium sulphate precipitated samples not for the dialyzed samples)

Unit definition: One unit of Ceruloplasmin is the amount of enzyme that will oxidize one µmol of Substrate per min. at 25°C.

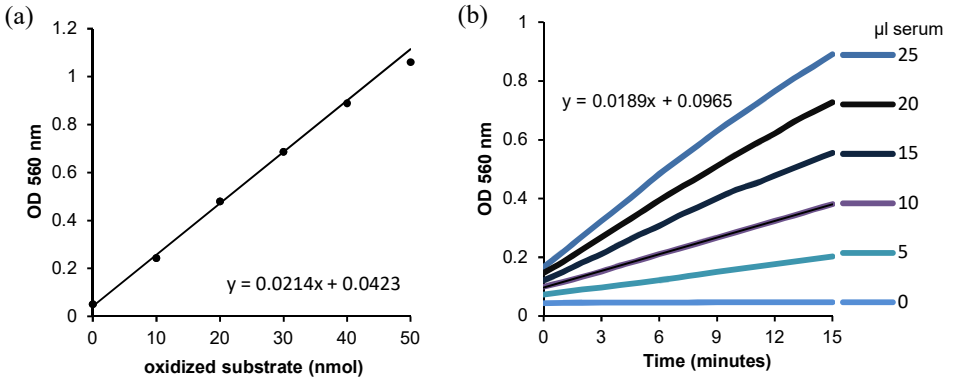

Figure: (a) Standard Curve of chemically oxidized substrate. (b) Ceruloplasmin Activity Assay: Kinetic curves obtained from varying sample volumes of human serum. Assays were performed following kit protocol. The Standard Curve slope (for the points below 1.0 OD) is 0.0214 (a) & the kinetic slope for a 10 µl sample of an Ammonium Sulfate precipitate of frozen pooled human serum is 0.0189 (b). Serum Ceruloplasmin Activity = 0.0189 (OD/min)/0.0214 (OD/nmol)/0.01ml X 2 = 176 mU/ml.

FOR RESEARCH USE ONLY! Not to be used on humans.