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Product Information

Ceruloplasmin Activity Colorimetric Kit

Catalog Number **MAK177**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

Ceruloplasmin is a multifunctional copper-containing ferroxidase present in serum. Ceruolpasmin is the major transporter of copper in the blood, carrying more than 95% of the copper found in plasma. It uses copper to couple the oxidation of substrate with the reduction of oxygen to water. Ceruloplasmin-mediated oxidation of ferrous (Fe^{2+}) iron to ferric (Fe^{3+}) iron contributes to the regulation of iron efflux in cells that have transportable iron stores. Inherited loss-of-function mutations in ceruloplasmin has been linked to aceruloplasminemia.[1] Ceruloplasmin is known to form complexes with cationic proteins during inflammation and is also involved in preventing iron-induced oxidative damage.[2] Increased ceruloplasmin levels have been observed in celiac and Crohn's patients.[3] Elevated serum ceruloplasmin has also been linked to cancer, pregnancy, rheumatoid arthritis, schizophrenia, and Alzheimer's disease, whereas its low levels have been linked to Wilson's and Menkes' diseases. Hence, measurement of ceruloplasmin activity in biological samples can provide useful insights into several metabolic and pathological conditions.

The Ceruloplasmin Activity Colorimetric Kit provides a fast and easy-to-use procedure for measuring ceruloplasmin activity in serum. Ceruloplasmin activity is determined using substrate oxidation, which results in a colorimetric (560 nm) product proportional to the enzymatic activity present. One unit of ceruloplasmin is the amount of enzyme that will oxidize 1 μmole of substrate per minute at 25 °C.

Components

The kit is sufficient for 100 assays in 96 well plates.

Ceruloplasmin Assay Buffer Catalog Number MAK177A	25 mL
Ammonium Sulfate, saturated (~4.1 M) Catalog Number MAK177B	10 mL
Ceruloplasmin Substrate Catalog Number MAK177C	1 mL
Oxidizer (100 mM) Catalog Number MAK177D	100 μL
Stabilizer Catalog Number MAK046E	100 μL

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use clear plates for colorimetric assays.
- Spectrophotometric multiwell plate reader

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening.

Ceruloplasmin Assay Buffer – Place the buffer into a water bath set at 25 °C for 30 minutes before use.

All other components are ready-to-use as supplied.

Storage/Stability

The kit is shipped on wet ice and storage at 2–8 °C, protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate.

Standards for Colorimetric Detection
For this assay, the standard curve is generated by non-enzymatic oxidation using the chemical Oxidizer. The Stabilizer protects product color for up to 15 minutes, hence it is recommended to read the Standard Curve within that time. This timeframe is also compatible for measuring the enzyme activity.

Dilute 10 µL of the 100 mM Oxidizer with 180 µL of the Ceruloplasmin Assay Buffer and 10 µL of Stabilizer, and mix well to prepare a 5 mM standard solution. Add 0, 2, 4, 6, 8, and 10 µL of the 5 mM Oxidizer into a 96 well plate, generating 0 (blank), 10, 20, 30, 40, and 50 nmole/well Standards. Add Ceruloplasmin Assay Buffer to each well to bring the volume to 100 µL.

Sample Preparation
Serum samples contain chloride, which inhibits ceruloplasmin activity and hence chloride should be removed from serum samples before use.

For removal of chloride from serum, add 100 µL of the saturated Ammonium Sulfate solution to 100 µL of serum and vortex briefly. Place on ice for 5 minutes. Centrifuge at 10,000 rpm at room temperature for 5 minutes to pellet proteins. Pipette out 160 µL of the clear supernatant. Add 160 µL of water to dissolve the pellet.

Alternatively, the serum sample can be dialyzed against 1,000× volume of water for 1 hour to remove chloride.

Add 5–20 µL of chloride-free serum samples into 96 well plate. Bring samples to a final volume of 100 µL with Ceruloplasmin Assay Buffer.

Assay Reaction
1. Set up the Reaction Mix according to the scheme in Table 1. 100 µL of the Reaction Mix is required for each reaction (well).

Table 1.
Reaction Mix

Reagent	**Standards and Samples**
Ceruloplasmin Assay Buffer	90 µL
Ceruloplasmin Substrate	10 µL

2. Add 100 µL of the Reaction Mix to each of the sample and standard wells. Mix well using a horizontal shaker or by pipetting.

3. Set the plate reader to 25 °C. In kinetic mode, measure A_{560} for 15 minutes for the Samples. Protect the plate from light. The Standards can be read in endpoint mode at the end of incubation time.

   Notes: Cold samples (not adequately equilibrated to 25 °C) will cause a slight lag phase detectable for the first 1–2 minutes.

   Ceruloplasmin catalyzes a one electron oxidation of the substrate resulting in the red product. This product is increasingly unstable as its concentration increases. Two molecules of the red product undergo a disproportionation reaction to 1 molecule of substrate and 1 molecule of a 2 electron oxidized 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 reaction results in a slight downward bend of the enzymatic reaction after ~15 minutes as the A_{560} approaches 1.

   The reaction is linear for only 15–20 minutes and tends to slow slightly after that.

Results

Calculations

1. Use the A_{560} of the chemically oxidized standards at 15 minutes to plot the standard curve and then calculate the slope of the curve (S_S).
   Notes: A new standard curve must be set up each time the assay is run.

   For accurate results, use A_{560} values <1 to calculate the slope of the standard curve.

   Slope (S_S) = A_{560}/nmole of oxidized substrate

2. Determine the linear range of the curve for each sample. Calculate the kinetic slope for each sample in the linear range (S_K).

   $$S_K = \Delta A_{560}/\text{minute} = [(A_{560})_2 - (A_{560})_1]/(T_2 - T_1)$$

   where:
   $(A_{560})_2$ is the absorbance at the end of the linear range
   $(A_{560})_1$ is the absorbance at the start of the linear range
   T_1 is the time at the start of the linear range
   T_2 is the time at the end of the linear range

3. Ceruloplasmin activity of a sample may be determined by the following equation:

   $$\text{Ceruloplasmin Activity} = \frac{S_K/S_S}{V \times 2}$$

   Where:
   S_K = kinetic slope of the sample (ΔA_{560}/minute) in the linear portion of the curve
   S_S = slope of the standard curve (A_{560}/nmole)
   V = sample volume (mL) added to well
   2 = Sample dilution factor for ammonium sulphate precipitated samples (not necessary for dialyzed samples).

One unit of ceruloplasmin is the amount of enzyme that will oxidize 1 μmole of substrate per minute at 25 °C.

Ceruloplasmin activity is reported as mU/mL or U/L.

References

1. Hellman, N.E. et al., Ceruloplasmin metabolism and Function. Ann. Rev. Nutr., **22**, 439-458 (2002).
2. Samygina, V.R. et al., Ceruloplasmin: macromolecular assemblies with iron-containing acute phase proteins. PLoS One, **8(7)**, e67145 (2013).
3. Ince, A.T. et al., Serum copper, ceruloplasmin and 24-h urine copper evaluations in celiac patients. Dig. Dis. Sci., **53(6)**, 1564-1572, (2008).

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at 25 °C
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Master Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Master Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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