Ceruloplasmin Colorimetric Activity Kit

Catalog Number EIACPLC (192 tests)

Rev 1.0

For safety and biohazard guidelines, see the "Safety" appendix in the *ELISA Technical Guide* (Pub. no. MAN0006706). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The Ceruloplasmin Colorimetric Activity Kit is an activity assay designed to measure ceruloplasmin activity in serum and urine samples. The assay was characterized with human ceruloplasmin, but is expected to measure ceruloplasmin activity in samples from other species.

Ceruloplasmin is a multi-copper oxidase enzyme that is part of certain vertebrate metabolic pathways. Ceruloplasmin contains 95% of the copper in serum. It is expressed in the liver, brain, lung, spleen, and testis.

Contents and storage

Kit and components are shipped at –20°C. Upon receipt, store the kit at –20°C. Once open, store the kit at 4°C and use within 2 weeks. Store the Ceruloplasmin Standard at –20°C after opening.

Components	Quantity
Ceruloplasmin Standard; 200 U/mL ceruloplasmin from human blood in a special stabilizing solution	20 µL
Assay Buffer Concentrate (5X)	28 mL
Clear 96-well Half Area Plates	2 plates
Ceruloplasmin Colorimetric Substrate; lyophilized substrate	2 vials
Plate Sealer	2

Materials required but not supplied

- Distilled or deionized water
- Microtiter plate reader with software capable of measurement at or near 560 nm
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solution
- 30°C incubator

Procedural guidelines

Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

Prepare 1X Assay Buffer

1. Dilute 14 mL of Assay Buffer (5X) with 56 mL of deionized or distilled water. Label as 1X Assay Buffer.
2. Store the concentrate and 1X Assay Buffer in the refrigerator. 1X Assay Buffer is stable at 2°C to 8°C for 3 months.

Sample preparation guidelines

- Collect samples in pyrogen/endotoxin-free tubes.
- Freeze samples after collection if samples will not be tested immediately. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well (do not vortex) prior to analysis.
- Avoid the use of hemolyzed or lipemic sera.
- If large amounts of particulate matter are present in the sample, centrifuge or filter sample prior to analysis.

Dilute samples

Sample concentrations should be within the range of the standard curve. Because conditions may vary, each investigator should determine the optimal dilution for each application.

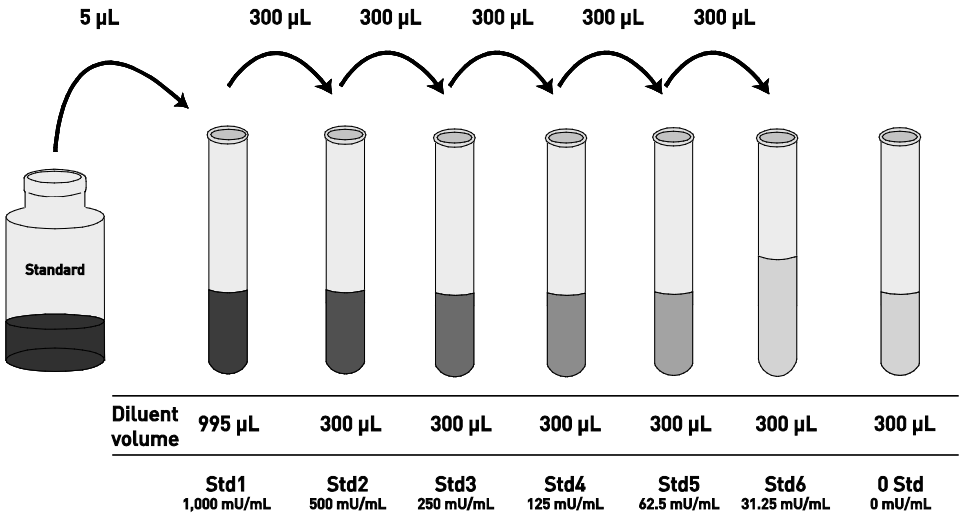
- Dilute **serum** samples ≥1:20 in 1X Assay Buffer.
- Dilute **urine** samples ≥1:20 in 1X Assay Buffer. For reporting urinary ceruloplasmin activity, use the Creatinine Urinary Assay Kit (Cat. No. EIACUN) to normalize to urine volume.
- Use all samples within **2 hours** of dilution.

Dilute standards

Note: Use glass or plastic tubes for diluting standards.

Note: One unit of ceruloplasmin activity is arbitrarily defined as the activity of an amount of ceruloplasmin resulting in an OD of 0.10 at 550 nm under defined conditions. The activity of this standard is defined using the Curzon and Vallet method.

1. Add 5 µL Ceruloplasmin Standard to one tube containing 995 µL 1X Assay Buffer and label as 1,000 mU/mL ceruloplasmin.
2. Add 300 µL 1X Assay Buffer to each of 6 tubes labeled as follows: 500, 250, 125, 62.5, 31.25, and 0 mU/mL ceruloplasmin.
3. Make serial dilutions of the standard as described below in the dilution diagram. Mix thoroughly between steps.
4. **Use the standards within 2 hours of preparation**.



Reconstitute Ceruloplasmin Substrate

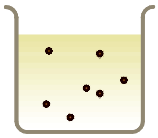
1. Add 3 mL of water to the vial of Ceruloplasmin Colorimetric Substrate and mix thoroughly.
2. Store this reconstituted Ceruloplasmin Substrate at 4°C for up to 2 weeks, or at –20°C for long term storage.

Assay procedure

Allow all reagents to reach room temperature before use. Mix all liquid reagents prior to use. **Total assay time is 60 minutes.**

IMPORTANT! Perform a standard curve with each assay.

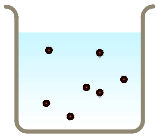
Determine the number of 8-well strips required for the assay. Insert the strips in the frames for use. Re-bag any unused strips and frames, and store at 2 to 8°C for future use.



Add sample

a. Pre-warm incubator to 30°C. [1]

b. Add 100 µL of standards or diluted samples (see page 2) to the appropriate wells.



Add substrate

a. Add 25 µL of the reconstituted Ceruloplasmin Substrate into each well.

b. Incubate for 60 minutes at 30°C. [1]

[1] Incubation at 37°C increases the optical density of the reaction by ~44%.

Incubation at room temperature (~21–23°C) decreases the optical density of the reaction by ~ 50% (the temperature used to determine this value was 22.3°C).

Read the plate and generate the standard curve

1. Read the absorbance at 560 nm.
2. Use curve-fitting software to generate the standard curve. A four parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.
3. Read the concentrations for unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.

   Note: Dilute samples producing signals greater than that of the highest standard in 1X Assay Buffer and reanalyze. Multiply the concentration by the appropriate dilution factor.

Performance characteristics

Standard curve (example)

The following data were obtained for the various standards over the range of 0–1,000 mU/mL ceruloplasmin.

Standard ceruloplasmin (mU/mL)	Optical Density (560 nm)
1,000	1.471
500	0.826
250	0.445
125	0.26
62.5	0.176
31.25	0.14
0	0.111

Intra-assay precision

Two panda urine samples were assayed 20 times in multiple assays to determine precision within an assay.

Parameters	Sample 1	Sample 2
Mean (mU/mL)	468.5	299.7
%CV	5.0	7.0

CV = Coefficient of Variation

Inter-assay precision

Two panda urine samples were assayed 16 times in duplicate assays in three independent trials to determine precision between assays.

Parameters	Sample 1	Sample 2
Mean (mU/mL)	480.6	285.4
%CV	9.6	18.8

CV = Coefficient of Variation

Performance characteristics, continued

Expected values

Human serum and urine samples from a variety of mammals (some pregnant), were tested in the assay.

Sample	Range (mU/mL)	Average (mU/mL)
Human serum (n=12)	6,934–28,254	17,859
Urine [1]	906–14,000	4,264

[1] After adjusting for urinary creatinine the normalized activity values ranged from 630 to over 35,000 mU/mg creatinine. Urinary creatinine concentrations were determined using the Creatinine Urinary Detection Kit (Cat. No. EIACUN).

Linearity of dilution

Linearity was determined by assaying panda urine or human serum samples with high and low concentrations of ceruloplasmin mixed in the ratios shown in the following table.

High Sample %	Low Sample %	Expected Conc. (mU/mL)		Observed Conc. (mU /mL)		% Recovery	
		Urine	Serum	Urine	Serum	Urine	Serum
80	20	535.0	463.4	564.6	507.4	105.5	90.5
60	40	439.3	428.3	450.7	426.8	102.6	111.0
40	60	343.7	393.2	400.2	436.4	116.5	99.6
20	80	248.0	358.1	246.9	324.4	99.5	109.5
				Mean Recovery		**106.0%**	**102.7%**

Sensitivity

The analytical sensitivity of the assay is 3.26 mU/mL ceruloplasmin. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding activity.

Limited product warranty

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Catalog Number

Batch code


Temperature limitation


Use by


Manufacturer


Consult instructions for use


Caution, consult accompanying documents

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