

KAMIYA BIOMEDICAL COMPANY

Ceruloplasmin Colorimetric Activity Kit

**For the quantitative determination of ceruloplasmin in
serum and urine samples**

Cat. No. KT-713

For Research Use Only.

PRODUCT INFORMATION

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BACKGROUND

Ceruloplasmin (Cp) is a multicopper oxidase enzyme involved in the safe handling of oxygen in some metabolic pathways of vertebrates. Discovered in 1948, a blue protein from the α 2-globulin fraction of human serum possessing oxidase activity towards aromatic diamines and catechol was purified by Holmberg and Laurell. It was denoted ceruloplasmin, literally meaning 'a blue substance from plasma'. Specialized copper sites have been recruited during evolution to provide long-range electron transfer reactivity and oxygen binding and activation in proteins destined to cope with oxygen reactivity in different organisms. Ceruloplasmin belongs to the family of multicopper oxidases which are among the few enzymes able to bind molecular oxygen to perform its complete reduction to water. Ceruloplasmin contains 95% of the copper in serum. Cp found in serum is expressed in the liver, but it is also expressed in the brain, lung, spleen and testis.

Aceruloplasminaemia is an autosomal recessive disorder of iron metabolism characterized by the complete absence of ceruloplasmin. The role of Cp in tissue iron overload and the subsequent clinical findings of diabetes, retinal degeneration and neurodegeneration has been associated with iron overload in aceruloplasminaemic patients. Thus it is clearly indicated that ceruloplasmin plays an essential role in iron metabolism. Ceruloplasmin is also associated with reproduction. Copper-deficient female rats seem to be protected against mortality. This protection has been suggested to be provided by estrogens, since estrogens alter the subcellular distribution of copper in the liver, an increase in plasma copper levels and subsequent ceruloplasmin synthesis.

PRINCIPLE

The Ceruloplasmin Activity Kit is designed to quantitatively measure ceruloplasmin activity in diluted serum and urine samples. Please read the complete kit insert before performing this assay. A human ceruloplasmin calibrator is provided to generate a calibration curve for the assay and all samples should be read off of the calibration curve. Samples are diluted in the provided Assay Buffer and added to the wells of a half area clear plate. The reconstituted Ceruloplasmin Substrate is added and the plate is incubated at 30°C for 60 minutes. The ceruloplasmin in the calibrators and samples reacts with the substrate to produce a colored product. The optical density is read at 560 nm. Increasing levels of ceruloplasmin in the samples causes an increase in the fuschia (pink-purple) product. The activity of the ceruloplasmin in the sample is calculated after making a suitable correction for any dilution, using software available with most plate readers. The results are expressed in terms of units of ceruloplasmin activity per mL.

COMPONENTS

Clear 96 well Half Area Plates 2 Plates

Ceruloplasmin Calibrator 20 μ L

1,000 Units/mL of human ceruloplasmin in a special stabilizing solution.

Assay Buffer Concentrate 28 mL

A 5X concentrate containing detergents and stabilizers.

Ceruloplasmin Colorimetric Substrate 2 Vials

Ceruloplasmin substrate lyophilized from a special stabilizing solution.

Plate Sealers 2 Each

STORAGE

This kit should be stored at -20°C until the expiration date of the kit.

Once opened the kit can be stored at 4°C up to the expiration date on the kit label, **except for the Ceruloplasmin Calibrator and reconstituted Ceruloplasmin Substrate, which must be stored at -20°C.**

OTHER MATERIALS REQUIRED

Repeater pipet with disposable tips capable of dispensing 25 µL.

An incubator capable of maintaining 30°C

96 well plate reader capable of reading optical density at 560 nm.

Software for converting optical density readings from the plate reader and carrying out four parameter logistic curve (4PLC) fitting. Contact your plate reader manufacturer for details.

PRECAUTIONS

As with all such products, this kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product.

The Ceruloplasmin Calibrator supplied in this kit is purified from human blood. The source of the protein was tested and found to be negative for hepatitis and HIV. However please treat the calibrator as a potentially infectious sample.

SAMPLE TYPES AND PREPARATION

Samples that need to be stored after collection should be stored at -70°C or lower, preferably after being frozen in liquid nitrogen. This assay has been validated for serum and urine samples. Samples containing visible particulate should be centrifuged prior to using.

Ceruloplasmins are ancient enzymes that should behave in a similar manner to the colorimetric substrate. It is believed that the assay will measure Cp activity from a wide range of sources. It is up to the end user to determine if their samples can be measured using this assay.

SAMPLE PREPARATION

Serum and Urine Samples

Serum and urine samples should be diluted at least 1:20 fold in the Assay Buffer supplied.

CALIBRATOR PREPARATION

Allow the kit reagents to come to room temperature for 30-60 minutes. We recommend that all calibrators and samples be run in duplicate to allow the end user to accurately determine Ceruloplasmin activities. Ensure that all samples have reached room temperature and have been diluted as appropriate prior to running them in the kit.

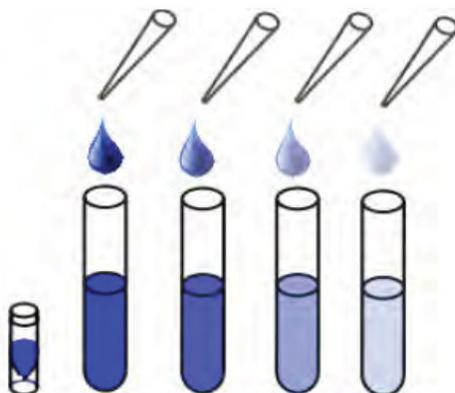
Assay Buffer

Dilute Assay Buffer Concentrate 1:5 by adding one part of the concentrate to four parts of deionized water. Once diluted this is stable at 4°C for 3 months.

Calibrator Preparation

Calibrators are prepared by labeling seven tubes as #1 through #7. Add 995 µL of Assay Buffer to tube #1. Pipet 300 µL of Assay Buffer into tubes #2 to #7. Carefully add 5 µL of the Ceruloplasmin Stock from the vial to tube #1 and vortex completely. Take 600 µL of the Cp solution in tube #1 and add it to tube #2 and vortex completely. Repeat the serial dilutions for tubes #3 through #7. The ceruloplasmin activity in tubes 1 through 8 will be 5, 3.33, 2.22, 1.48, 0.988, 0.658, and 0.439 U/mL.

Use all Calibrators within 2 hours of preparation.



	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7
Assay Buffer Vol (µL)	995	300	300	300	300	300	300
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Vol of Addition (µL)	5	600	600	600	600	600	600
Final Activity (U/mL)	5.0	3.33	2.22	1.48	0.988	0.658	0.439

Ceruloplasmin Substrate Preparation

Add 3 mL of water to the vial and mix thoroughly. This solution can be stored at 4°C for up to 2 weeks. The solution can also be stored at -20°C for up to the expiration date on the kit label.

ASSAY PROTOCOL

Use the plate layout sheet on the back page to aid in proper sample and standard identification. Set plate parameters for a 96-well Corning Costar 3695 plate.

1. Pre-warm incubator to 30°C.
2. Pipet 100 µL of diluted samples or appropriate calibrators into duplicate wells in the plate.
3. Pipet 100 µL of Assay Buffer into duplicate wells as the Zero calibrator.
4. Add 25 µL of the reconstituted Cp Substrate solution to each well using a repeater pipet.
5. Incubate at 30°C for 60 minutes.
6. Read the optical density generated from each well in a plate reader capable of reading at 560 nm.

CALCULATION OF RESULTS

Average the duplicate OD readings for each calibrator and sample. Create a calibration curve by reducing the data using the 4PLC fitting routine on the plate reader, after subtracting the mean OD for the zero calibrator. The sample activity obtained should be multiplied by the dilution factor to obtain neat sample values.

TYPICAL DATA

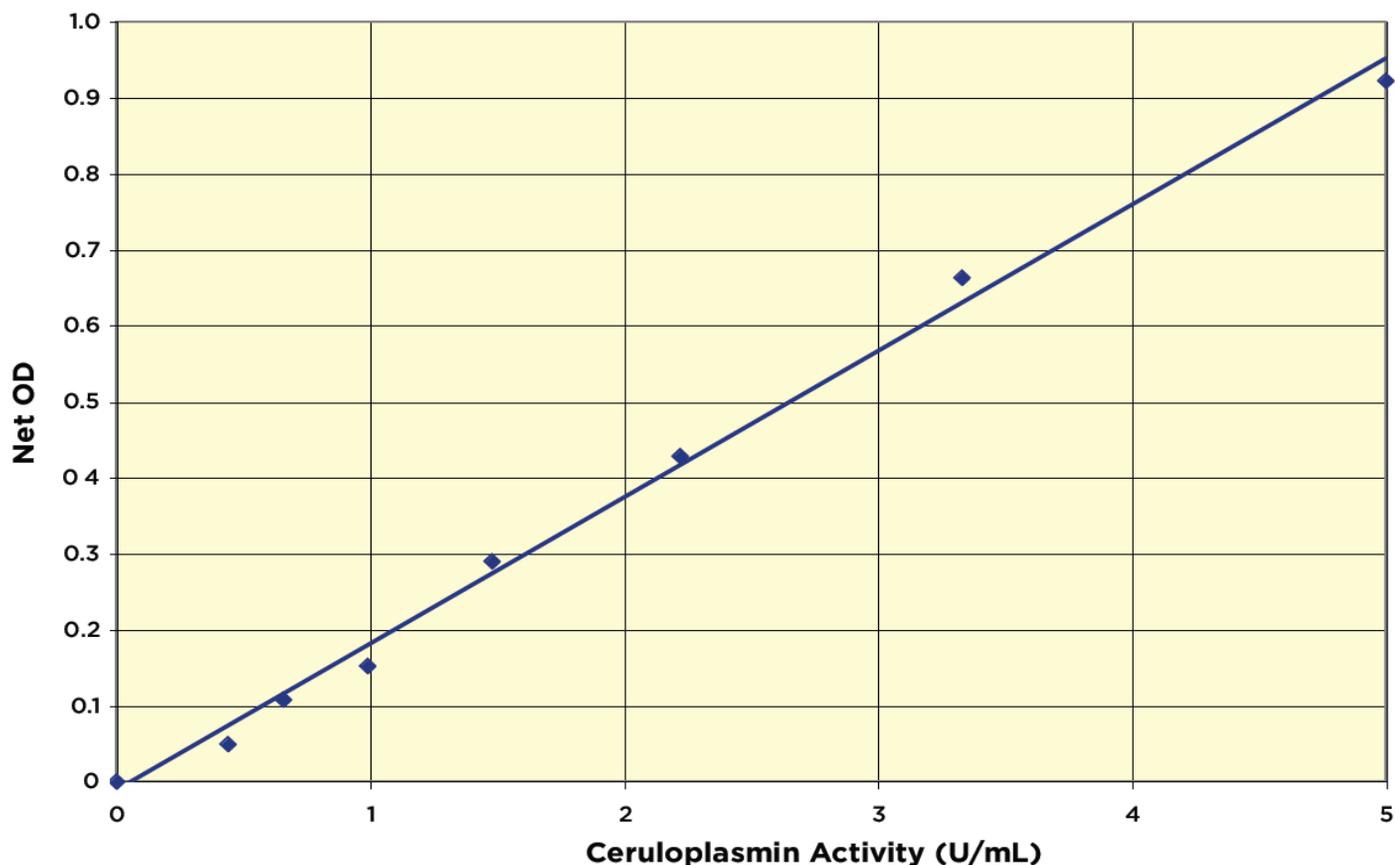
Sample	Mean Net OD	Ceruloplasmin Activity (U/mL)
Standard 1	0.922	5
Standard 2	0.663	3.33
Standard 3	0.428	2.22
Standard 4	0.290	1.48
Standard 5	0.152	0.988
Standard 6	0.108	0.658
Standard 7	0.049	0.439
Zero	0.000	0
Sample 1	0.337	1.831
Sample 2	0.209	1.169

Always run your own calibration curves for calculation of results. Do not use these data.

Ceruloplasmin Unit Definition

One Unit of Ceruloplasmin causes an increase in OD of 0.01 per minute at 37°C and pH 5.5 using N,N-dimethyl-p-phenylene diamine as substrate.

Typical Calibration Curve



Always run your own calibration curves for calculation of results. Do not use these data.

VALIDATION DATA

Sensitivity

Sensitivity was calculated by comparing the ODs for twenty wells run for each of the zero and calibrator #7. The detection limit was determined at two (2) standard deviations from the zero along the calibration curve.

Sensitivity was determined as 0.242 U/mL. This is equivalent to 24.2 mU/well.

Limit of Detection

The Limit of Detection for the assay was determined in a similar manner by comparing the ODs for twenty runs for each of the zero calibrator and a low concentration panda urine sample.

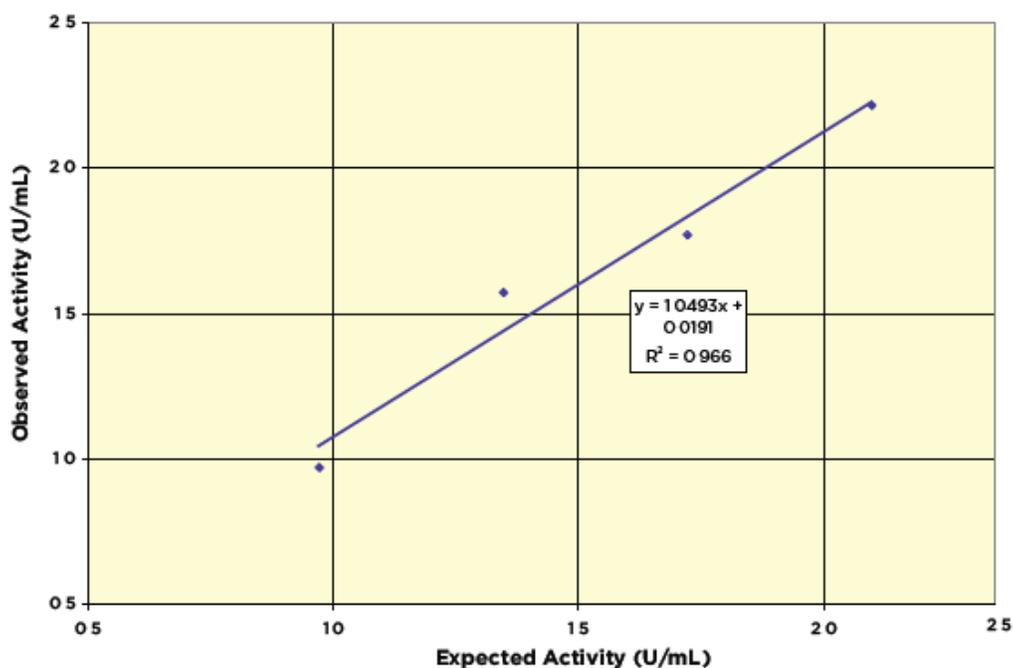
Limit of Detection was determined as 0.425 mU/mL. This is equivalent to 42.5 mU/well.

Linearity

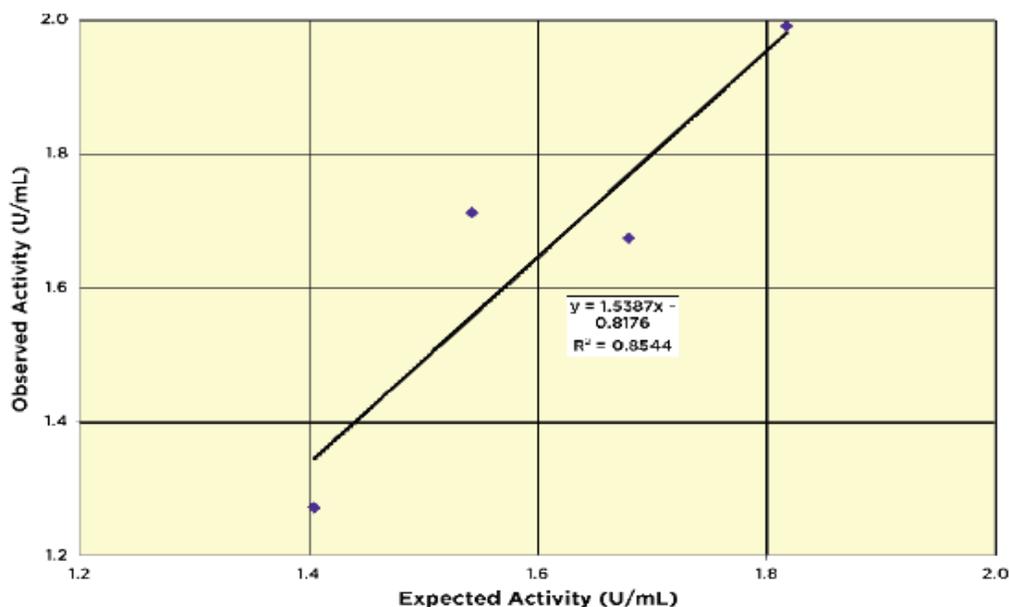
Linearity was determined by taking two diluted panda urine or human serum samples, one with a high known ceruloplasmin activity and the other with a lower ceruloplasmin activity and mixing them in the ratios given below. The measured activities were compared to the expected values based on the ratios used.

High Sample	Low sample	Expected Activity (U/mL)		Observed Activity (U/mL)		% Recovery	
		Urine	Serum	Urine	Serum	Urine	Serum
80%	20%	2.10	1.40	2.22	1.27	105.5	90.5
60%	40%	1.72	1.54	1.77	1.71	102.6	111.0
40%	60%	1.35	1.68	1.57	1.67	116.5	99.6
20%	80%	0.97	1.82	0.97	1.99	99.5	109.5
Mean Recovery						106.0%	102.6%

Urine Linearity



Serum Linearity



Intra Assay Precision

Two panda urine samples diluted in Assay Buffer were run in replicates of 20 in an assay. The mean and precision of the calculated activities were:

Sample	Ceruloplasmin Activity (U/mL)	%CV
1	1.85	5.0
2	1.17	7.1

Inter Assay Precision

Two panda urine samples diluted in Assay Buffer were run in duplicates in sixteen assays run over multiple days by three operators. The mean and precision of the calculated activities were:

Sample	Ceruloplasmin Activity (U/mL)	%CV
1	1.92	8.7
2	1.17	12.2

SAMPLE VALUES

Urine samples from a variety of mammals, some of them pregnant, were tested in the assay. Activity values, after adjustment for dilution, ranged from 11.26 U/mL to 73.7 U/mL, with an average of 29.6 U/mL. After adjusting for urinary creatinine the normalized activity values ranged from 11.3 to over 180 U/mg creatinine.

This assay has not yet been tested on fecal extracts or plasma samples.

Seven random human serum were diluted with Assays Buffer and tested in the assay. Activity values ranged from 57.01 to 128.4 U/mL with an average value of 87.9 U/mL.

FOR RESEARCH USE ONLY

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A												
B												
C												
D												
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