ARBOR ASSAYS™
Interactive Assay Solutions™

MULTI SPECIES

MOST SENSITIVE

DetectX®

Ceruloplasmin
Colorimetric Activity Kit

2 Plate Kit Catalog Number K035-H1

Species Independent

Sample Types Validated:

Serum and Urine

Protected by US Patent 8,227,206

Please read this insert completely prior to using the product.
For research use only. Not for use in diagnostic procedures.

www.ArborAssays.com

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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 α2-globulin fraction of human serum possessing oxidase activity towards aromatic diamines and catechol was purified by Holmberg and Laurell[1]. 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[2,3]. Ceruloplasmin contains 95% of the copper in serum[4]. 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[5,6]. 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[7]. 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[8].

1. Holmberg CG and Laurell CB. Investigation serum copper. II. Isolation of the copper containing protein, and a description of some its properties. 1948, Acta. Chem. Scand. 2:550–556.
2. Malmstrom BG. Enzymology of oxygen. Annu. Rev. Biochem. 1982, 51: 21–59.
3. Farver O. and Pecht I. Electron transfer reactions in multi-copper oxidases. In: Multi-Copper Oxidases, 1997, pp. 355–389, Messerschmidt A. (ed.), World Scientific Publication, Singapore.
4. Harris, ZL and Gitlin, JD, Genetic and molecular basis for copper toxicity, Am. J. Clin. Nutr. 1996, 63:836S–841S.
5. Yoshida K., . et al. A mutation in the ceruloplasmin gene is associated with systemic hemosiderosis in humans. Nat. Genet. 1995, 9:267–272.
6. Harris ZL., Takahashi Y., Miyajima H., Serizawa M., MacGillivray RTA. and Gitlin JD. Aceruloplasminemia: molecular characterization of a novel disorder of iron metabolism. Proc. Natl. Acad. Sci. USA, 1995, 92:2539–2543.
7. Takahashi Y., Miyajima H., Shirabe S., Nagataki S., Suenaga, A. and Gitlin JD. Characterization of a nonsense mutation in the ceruloplasmin gene resulting in diabetes and neurodegenerative disease. 1996, Hum. Mol. Genet. 5:81–84.
8. Fields, M., Lewis, C., Scholfield, D., Powel, AS., Rose, AJ., Reiser, S., and Smith, JC., Female rats are protected against the fructose-induced mortality of copper deficiency. Proc. Soc. Expl Biol. Med. 1986, I86:145-I49.

ASSAY PRINCIPLE

The DetectX® 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 standard is provided to generate a standard curve for the assay and all samples should be read off of the standard 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 standards 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 fuchsia (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 milliunits (mU) of ceruloplasmin activity per mL.

Ceruloplasmin activity is based upon the published determination by G. Curzon and L. Vallet, Biochem. J., 1960, 74:279-287. Due to our optimized reagents and incubation times the optical density generated by 1 U/mL of ceruloplasmin is substantially higher than 0.1 OD.

RELATED PRODUCTS

Kits	Catalog No.
17-Hydroxyprogesterone ELISA Kits	K053-H1/H5
Aldosterone ELISA & Chemiluminescent ELISA Kits	K052-H1/H5, K052-C1/C5
Allopregnanolone ELISA Kits	K061-H1/H5
Dehydro-epiandrosterone sulfate (DHEA-S) ELISA Kits	K054-H1/H5
Estradiol Non-Invasive & Serum ELISA Kits	K030-H1/H5, KB30-H1/H5
Estrone ELISA Kits	K031-H1/H5
Hemoglobin High Sensitivity Detection Kit	K013-HX1/HX5
Levonorgestrel ELISA Kits	K058-H1/H5
Nitric Oxide Colorimetric Detection Kit	K023-H1
Oxytocin ELISA & Chemiluminescent ELISA Kits	K048-H1/H5, K048-C1/C5
PGFM (13,14-dihydro-15-keto-Prostaglandin F2a) ELISA Kits	K022-H1/H5
Progestrone ELISA Kits	K025-H1/H5
Progestrone Metabolites ELISA Kits	K068-H1/H5
Prolactin ELISA Kit	K040-H1
Superoxide Dismutase (SOD) Activity Kit	K028-H1

SUPPLIED COMPONENTS

Clear Half Area 96 well Plates
Corning CoStar Plate 3695.

2 Plates	Catalog Number X018-2EA

Ceruloplasmin Standard*
200 Units/mL of human ceruloplasmin in a special stabilizing solution.

20 µL	Catalog Number C189-20UL

Assay Buffer Concentrate
A 5X concentrate containing detergents and stabilizers.

28 mL	Catalog Number X111-28ML

Ceruloplasmin Colorimetric Substrate
Ceruloplasmin substrate lyophilized from a special stabilizing solution.

2 Vials	Catalog Number C121-1EA

Plate Sealers

2 Each	Catalog Number X002-1EA

* Ceruloplasmin activity is based upon the published determination by G. Curzon and L. Vallet, Biochem. J., 1960, 74:279-287. The definition of a unit of activity was arbitrarily defined as the activity of an amount of ceruloplasmin giving an OD of 0.10 at 550 nm under their defined conditions. The activity of our standard is determined using the Curzon and Vallet method.

STORAGE INSTRUCTIONS

This kit must 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 Standard 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 Standard 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 standard 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 in the diluted Assay Buffer.

For reporting urinary Ceruloplasmin Activity values, use our DetectX® Urinary Creatinine Detection Kits, K002-H1 and K002-H5, to normalize to urine volume.

REAGENT PREPARATION

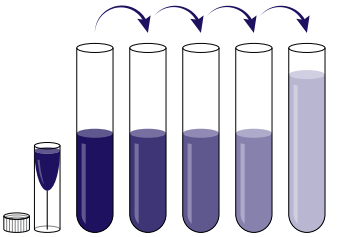
Allow the kit reagents to come to room temperature for 30-60 minutes. Ensure that all samples have reached room temperature and have been diluted as appropriate prior to running them in the kit.

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.

Standard Preparation

Standards are prepared by labeling tubes as #1 through #7. Add 995 µL of Assay Buffer to tube #1. Pipet 300 µL of Assay Buffer into tubes #2 to #6. Carefully add 5 µL of the Ceruloplasmin Stock from the vial to tube #1 and vortex completely. Take 300 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 #6. The ceruloplasmin activity in tubes 1 through 8 will be 1,000, 500, 250, 125, 62.5, and 31.25 mU/mL.



Use all Standards within 2 hours of preparation.

	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Assay Buffer Vol (µL)	**995**	300	300	300	300	300
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5
Vol of Addition (µL)	**5**	300	300	300	300	300
Final Activity (mU/mL)	1,000	500	250	125	62.5	31.25

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

We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine Ceruloplasmin activities.

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. See: www.ArborAssays.com/resources/#general-info for plate dimension data.

1. Pre-warm incubator to 30°C.
2. Pipet 100 µL of diluted samples or appropriate standards into duplicate wells in the plate.
3. Pipet 100 µL of Assay Buffer into duplicate wells as the Zero standard.
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.

NOTES

37°C Incubation

Incubation at 37°C will increase the optical density of the reaction by about 44%.

Room Temperature Incubation

Incubation at room temperature (~21-23°C) will decrease the optical density of the reaction by about 50%. This measurement was made with room temperature at 22.3°C.

CALCULATION OF RESULTS

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

Or use the online tool from MyAssays to calculate the data:
https://www.myassays.com/arbor-assays-ceruloplasmin-colorimetric-activity-kit-k035.assay

TYPICAL DATA

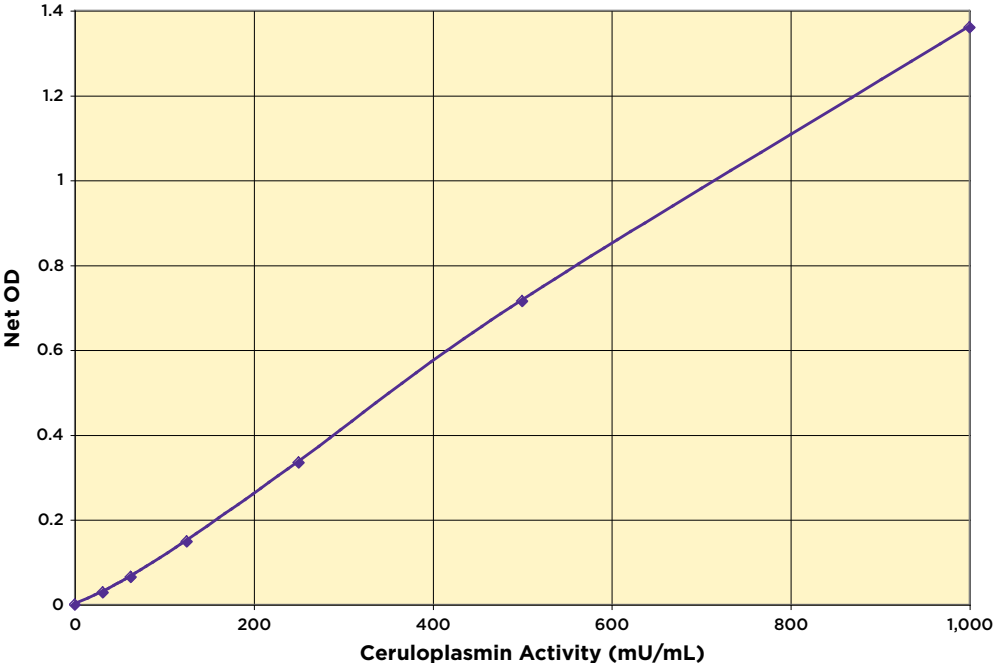
Sample	Mean OD	Mean Net OD	Ceruloplasmin Activity (mU/mL)
Standard 1	1.471	1.36	1,000
Standard 2	0.826	0.715	500
Standard 3	0.445	0.335	250
Standard 4	0.26	0.149	125
Standard 5	0.176	0.065	62.5
Standard 6	0.14	0.029	31.25
Zero	0.111	0	0
Sample 1	0.767	0.657	460.6
Sample 2	0.488	0.378	277.6

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

Ceruloplasmin Unit Definition

Ceruloplasmin activity is based upon the published determination by G. Curzon and L. Vallet, Biochem. J., 1960, 74:279-287. The definition of a unit of activity is arbitrarily defined as the activity of an amount of ceruloplasmin giving an OD of 0.10 at 550 nm under the published defined conditions.

Typical Standard Curve



Always run your own standard 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 standard #6. The detection limit was determined at two (2) standard deviations from the zero along the standard curve.

Sensitivity was determined as 3.26 mU/mL. This is equivalent to 0.326 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 standard and a low concentration panda urine sample.

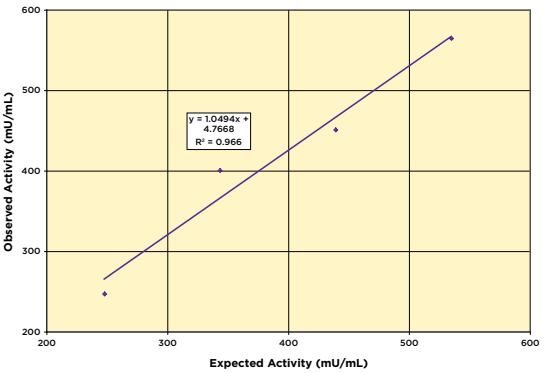
Limit of Detection was determined as 2.17 mU/mL. This is equivalent to 0.217 mU/well.

Linearity

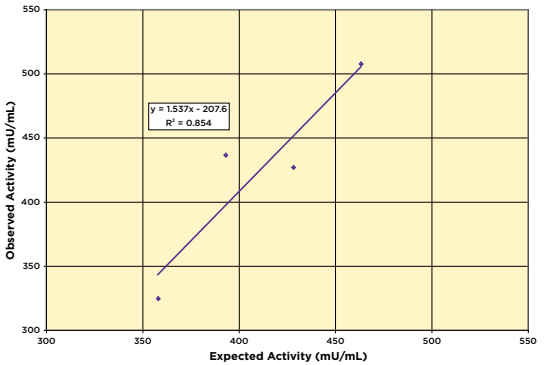
Linearity was determined by taking two diluted panda urine samples, one with a high known ceruloplasmin activity and the other with a lower ceruloplasmin activity, and mixing them in the ratios given below. Linearity for human serum samples was determined in a similar manner. The measured activities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

		Expected Activity (mU/mL)		Observed Activity (mU/mL)		% Recovery	
High Sample	Low sample	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%**

Urine Linearity



Serum Linearity



Intra Assay Precision

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

Sample	Ceruloplasmin Activity (mU/mL)	%CV
1	468.5	5.0
2	299.7	7.0

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

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 906 mU/mL to over 14,000 mU/mL, with an average of 4,264 mU/mL. 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 Arbor Assays DetectX® Kits K002-H1 and K002-H5.

Twelve random human serum were diluted with Assays Buffer and tested in the assay. Adjusted activity values ranged from 6,934 to 28,254 mU/mL with an average value of 17,859 mU/mL.

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

LIMITED WARRANTY

Arbor Assays warrants that at the time of shipment this product is free from defects in materials and workmanship. This warranty is in lieu of any other warranty expressed or implied, including but not limited to, any implied warranty of merchantability or fitness for a particular purpose.

We must be notified of any breach of this warranty within 48 hours of receipt of the product. No claim shall be honored if we are not notified within this time period, or if the product has been stored in any way other than outlined in this publication. The sole and exclusive remedy of the customer for any liability based upon this warranty is limited to the replacement of the product, or refund of the invoice price of the goods.

CONTACT INFORMATION

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OFFICIAL SUPPLIER TO ISWE

Arbor Assays and the International Society of Wildlife Endocrinology (ISWE) signed an exclusive agreement for Arbor Assays to supply ISWE members with assay kits and reagents for wildlife conservation research.

ASSAYS™
FOR SERIOUS SCIENCE

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