



**Ceruloplasmin (Cp)
Colorimetric Assay kit
(96 Tests)**

Zellbio GmbH (Germany)

CAT No. ZX-44115-96

www.zellx.de

Sample Types Validated for:

Serum, and Urine

!!! Caution: This product is for Research Use Only. Not for *in-vitro* Diagnostics !!!

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Please read this insert completely prior to using the product.

Introduction

Background

Ceruloplasmin (Cp) 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 literally meaning 'a blue substance from plasma', is a blue copper-binding (6-7 atoms per molecule) glycoprotein from the α_2 -globulin fraction of human serum possessing oxidase activity towards aromatic diamines and catechol. The Ceruloplasmin found in the serum is mainly produced in liver, but it is also expressed in brain, lung, spleen and testis. Ceruloplasmin contains 95% of the copper in serum, is involved in the safe handling of oxygen in several metabolic pathways in vertebrates, and is associated with reproduction. 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.

Aceruloplasminaemia is an autosomal recessive disorder of iron metabolism characterized by the complete absence of Ceruloplasmin. Due to the essential role of Ceruloplasmin in iron metabolism, aceruloplasminaemic patients suffer from iron overload in tissues, and subsequent clinical complications including diabetes, retinal degeneration and neurodegeneration.

Assay principle

The ZellX[®] Ceruloplasmin Activity Kit has been 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 the standard curve. Samples are diluted in the provided Assay Buffer, and added to the wells of a clear half area 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 fuchsia-colored (pink-purple) product. The optical density is read at 560 nm. Increasing levels of Ceruloplasmin in the samples results in an increase in the density of the colored product. The activity of the Ceruloplasmin in the sample is calculated after making a suitable correction for any dilution, using a software available in most plate readers. The results are expressed in terms of milliunits (mU) of Ceruloplasmin activity per milliliter (mL).

General information

Materials supplied in the Kit

| <i>Component</i> | <i>Quantity</i> |
|---|-----------------|
| Ceruloplasmin Standard (200 Unit/mL) | 10 µL |
| Assay Buffer Conc. | 14 mL |
| Ceruloplasmin Substrate | 1 vial |
| Clear Half Area 96 Well Plate | 1 plate |
| Plate sealer | 1 sealer |

Storage instruction

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.**

Materials required but not supplied

Deionized water (diH₂O)

Microplate/ELISA reader capable of reading optical absorption at 560 nm

Precision pipettes, multichannel/repeater pipettes and disposable pipette tips

An incubator capable of maintaining 30°C

Precautions

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 with this kit has been derived from human blood and should be treated as potentially infectious. Although the source of blood has been tested negative for Hepatitis B and C, and HIV, appropriate precautions should be taken.

General remarks

- The instruction must be strictly followed. The reading of Microplate/ELISA reader must be set at the appropriate wavelength for determining the experiment results.

- Pipette tips should not be used more than once to prevent cross contamination.
- Reagents of different batches should not be mixed or used after their expiration dates.

Assay protocol

Reagent preparation

Assay Buffer: Prepare a 1:5 dilution of Assay Buffer Concentrate with diH₂O (1 part Assay Buffer Conc. with 4 parts diH₂O), and mix well. Assay Buffer can be stored at 4°C for up to 3 months.

Ceruloplasmin Substrate Working Solution: Add 3 mL of diH₂O to the Ceruloplasmin Substrate 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 until the expiration date of the kit.

Sample preparation

Since Ceruloplasmins are ancient enzymes, they behave in a similar manner to the colorimetric substrate, it is expected that this kit can measure Ceruloplasmin in human and other species.

Samples containing visible particulate should be centrifuged prior to conducting the assay.

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

To report the urinary Ceruloplasmin Activity values, you may normalize the sample values based on the Creatinine levels using our Urine Creatinine assay kit Cat NO. ZX-44110-96 in a random urine specimen.

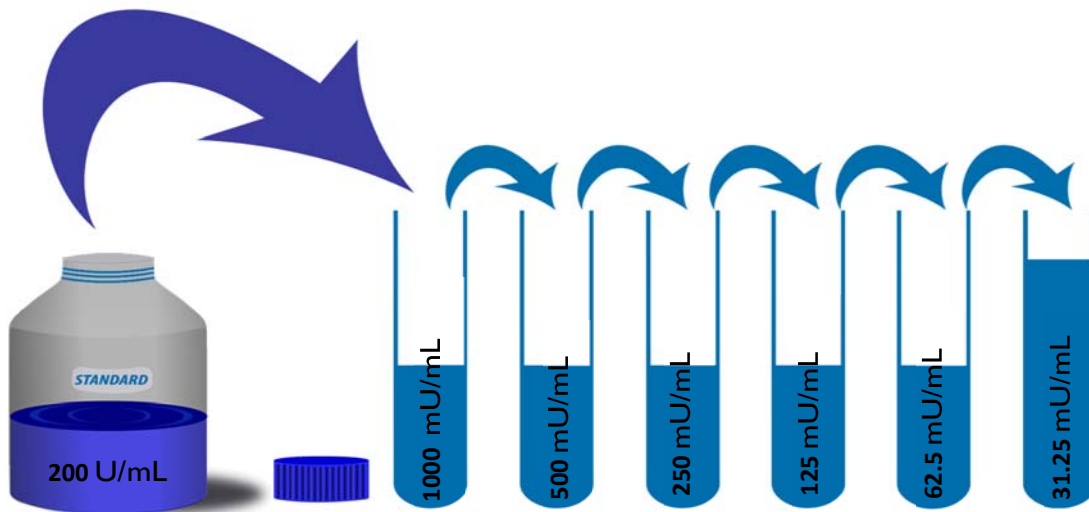
All the samples must be used within 2 hours of dilution or stored at -70°C or lower, preferably after being frozen in liquid nitrogen.

Standard preparation

For Normal protein concentration

- Prepare a 1:200 dilution of Cp Standard with Assay Buffer (mix 5 μ L of standard with 995 μ L of Assay Buffer), and label as the Standard No.6 (1000 mU/mL).
- Make series of lower dilutions as described in the table.
- The Assay Buffer is used as the 0 mU/mL standard.

| No. | Concentration | Material needed |
|---------------|---------------|--|
| Standard No.6 | 1000 mU/mL | 5 μ L Cp Standard + 995 μ L Assay Buffer |
| Standard No.5 | 500 mU/mL | 250 μ L Standard No.6 + 250 μ L Assay Buffer |
| Standard No.4 | 250 mU/mL | 250 μ L Standard No.5 + 250 μ L Assay Buffer |
| Standard No.3 | 125 mU/mL | 250 μ L Standard No.4 + 250 μ L Assay Buffer |
| Standard No.2 | 62.5 mU/mL | 250 μ L Standard No.3 + 250 μ L Assay Buffer |
| Standard No.1 | 31.25 mU/mL | 250 μ L Standard No.2 + 250 μ L Assay Buffer |
| Standard No.0 | 0 mU/mL | 250 μ L Assay Buffer |



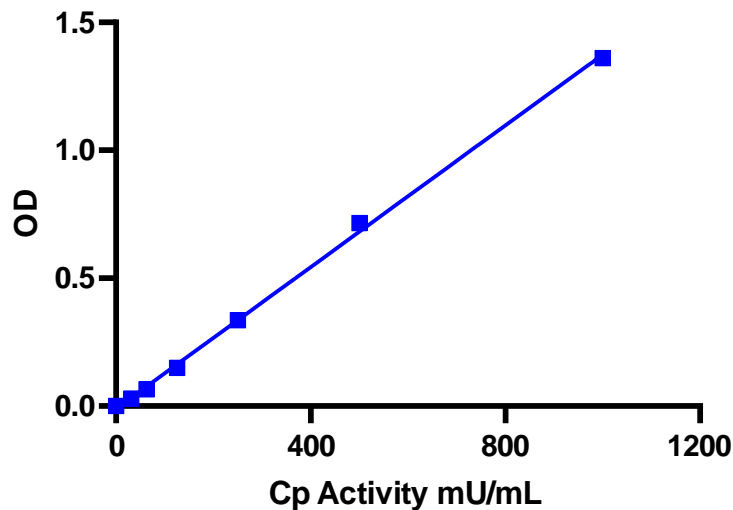
All standard must be used within 2 hours of preparation

Assay Procedure

1. Pre-warm incubator to 30°C.
2. Pipette 100 µL of either samples or standards into duplicate wells in the plate.
3. Pipette 100 µL of Assay Buffer into duplicate wells as the Zero standard.
4. Add 25 µL of Ceruloplasmin Substrate Working Solution to each well using a multichannel/repeater pipette.
5. Incubate at 30°C for 60 minutes. **Incubation at 37°C increases the optical density of the reaction by about 44%; whereas, incubation at room temperature (~21-23°C) decreases the optical density of the reaction by about 50%.**
6. Read the optical density at 560 nm.

Calculation

- Average the duplicate optical density (OD) readings for each standard and sample.
- Create a standard curve by reducing the data using the four parameter logistic curve (4PLC) fitting routine on the plate reader using the adjusted OD values
- The concentrations obtained should be multiplied by the dilution factor to obtain sample values.



A typical standard curve of ZELLX® Ceruloplasmin Assay kit

Run your own standard curves for calculation of results

Ceruloplasmin Unit definition: 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 (9).

Assay range

The limit of detection of ZellX® Ceruloplasmin assay was determined as 2.17 mU/mL.

Sensitivity

The sensitivity of the ZellX® Ceruloplasmin assay was determined as 3.26 mU/mL.

Precision

Intra-Assay Precision (Precision within an assay): 3 samples were tested 20 times in an assay.

Inter-Assay Precision (Precision between assays): 3 samples were tested in duplicate on 16 different assays over multiple days.

| <i>Item</i> | <i>%CV</i> |
|--------------------|------------|
| Intra assay | 7.0, 5.0 |
| Inter assay | 9.6, 18.8 |

Protocol summary

Add 100 µL samples/standard into duplicate wells



Add 100 µL Assay Buffer into duplicate wells as zero



Add 25 µL of the Ceruloplasmin Substrate Working Solution



Incubate at 30°C for 1 hours



Read the absorbance at 560 nm

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