# CUPRAC Assay Kit

## *KF01005*

## *250 tests (96 well plate)*

BQCkit

A brand of Bio QuChem

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All chemicals should be handled with care

- **This kit is for R&D use only**

# *Introduction*

Free radicals are highly reactive species that are naturally formed during cell metabolism in the mitochondria, secretion by immune cells during inflammatory processes or taken up as environmental pollutants.

CUPRAC (CUPric Reducing Antioxidant Capacity) is a mostly new method which enables the Total Antioxidant Capacity (TAC) measurements hydrophilic as well as hydrophobic samples. The main reagent, the copper (II)-neocuproine (2,9-dimethyl-1,10-phenanthroline), is able to oxidize antioxidants generating a coloured product. The chelation with neocuproine enables a fastened reaction by elevating the redox potential of the reagent.

The **BQC** CUPRAC Antioxidant Capacity Assay Kit is a stable, selective, easy and quick assay that measures all of the significant antioxidants on biological samples at physiological pH.

# *Materials*

BQCkit CUPRAC Antioxidant Capacity Assay kit ***KF01005-250 tests*** contain:

| Product | Quantity | Storage |
|---|---|---|
| **Reagent A** | 2 bottles | 4ºC |
| **Reagent B** | 2 bottles (powder) | 4ºC |
| **Reagent C** | 2 bottles | 4ºC |
| **Reagent D** | 1 bottle | 4ºC |
| **Reagent E** | 1 bottle | 4ºC |
| **Trolox Standard** | 2 vials (powder) | 4ºC |

# *Assay Principle*

On the CUPRAC assay, a redox reduction between the CUPRAC reagent and the antioxidants with a leading thiol group (like for example glutathione) present in the sample, takes place. In this process, the reagent reduces itself forming a chelate complex of copper (I) – neocuproine, which provides a colour measurable at 450 nm in a spectrophotometer.

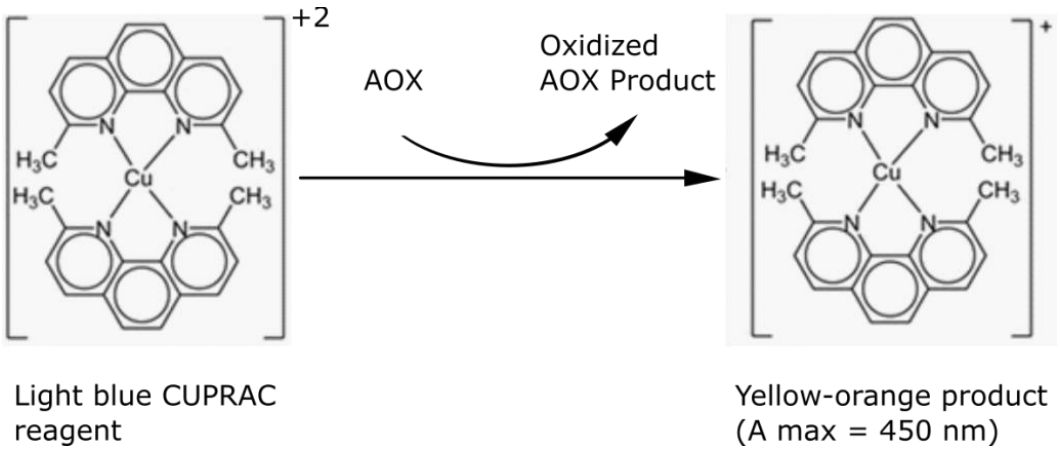


**Figure 1. CUPRAC Reagent reaction**

## *Reagent Preparation*

### Solution B:

Add exactly 5 mL of Reagent E to the vials of Reagent B that are going to be used immediately and mix well.

### Standard solution:

Add exactly 1 ml of Reagent E to the Trolox Standard vial and mix well. Prepare the calibration curve in 1 mL tubes as shown below. Antioxidant activity is expressed as µM Trolox Equivalents.

**Table1.** *Reagent volumes needed to carry out the standard curve.*

| Standard [µL] | Reagent E [µL] | Trolox [µM] |
|---|---|---|
| 0 | 500 | 0 |
| 12.5 | 487,5 | 0.25 |
| 25 | 475 | 0.5 |
| 50 | 450 | 1 |
| 75 | 425 | 1.5 |
| 100 | 400 | 2 |

### Plate Set up:

This scheme is just a recommendation on how to perform the assay. For optimal results, it is recommended to run the standards and the samples at least for duplicate, but it is the user´s discretion to do so.

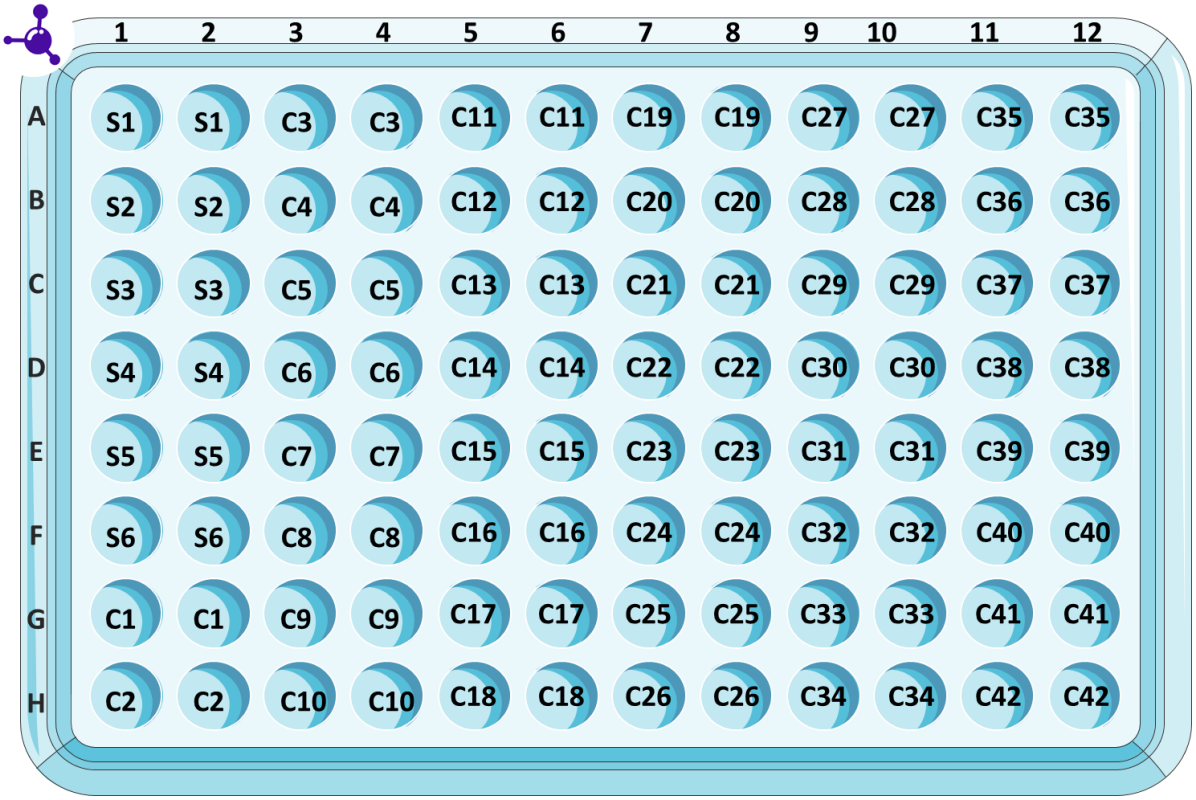


**Figure 2: S1-S6: Standards, C1-C42: Samples**

# *Sample Preparation*

Dilute your sample to an absorbance value corresponding to 0.75-1.5 µM of standard approximately. Filter your sample if it has solid particles in suspension as it might be the case for tea and juices. Store the samples frozen until the day of the assay and avoid contact with air, light and heat. Avoid the formation of bubbles and high speed with the vortex mixer to minimize the oxygen exposure.

## Sample blank

It is recommended to run a sample blank when the sample shows absorbance at 450 nm. Follow the Assay Protocol for the sample blank as indicated in steps 2 and 3.

## Wine

Recommended dilution is 1:100 with water.

## Plasma

Mix 60 µl of plasma with 120 µl of Reagent D

Vortex and centrifuge at 5000 rpm for 10 minutes

Collect approximately 50-60 µl of supernatant to assay or freeze

Dilution of plasma is usually not required. This protocol should be repeated for every replicate, with a different microtube for each.

# *Assay Protocol*

Invert the bottles a few times to ensure the reagents are well mixed before running the assay.
Do not run the standard curve and the samples at different times and do not reuse the calculations of another day. Keep the standard and the samples on the assay for the same amount of time. It is recommended to use a multi-channel pipette if possible.

1 

Set up the plate design and add 40 µl of sample/standard.

2 

Add 40 µl of Reagent A to each well **of standard and sample**. Add 40 µl of Reagent C to each **sample blank** well instead.

3 

Add 40 µl of Solution B previously prepared (See *Reagent Preparation* section) to each well **of standard and sample**. Add 40 µl of Reagent C to each **sample blank** well instead. Now, your standard should become yellow-orange.

4 

Add 40 µl of Reagent C to each well

5 

Add 40 µl of double distilled $H_2O$

6 

Let the reaction run for exactly 30 minutes.

7 

Read the absorbance at 450 nm.

# *Data Analysis*

## Analysis of the Standard:

1. If the spectrophotometer or microplate reader was not zeroed with the blank, then average the blank values and subtract the average blank value from the standard and unknown sample values.

2. Create a standard curve by plotting A 450 nm (y-axis) vs. standard, µM Trolox (x-axis).

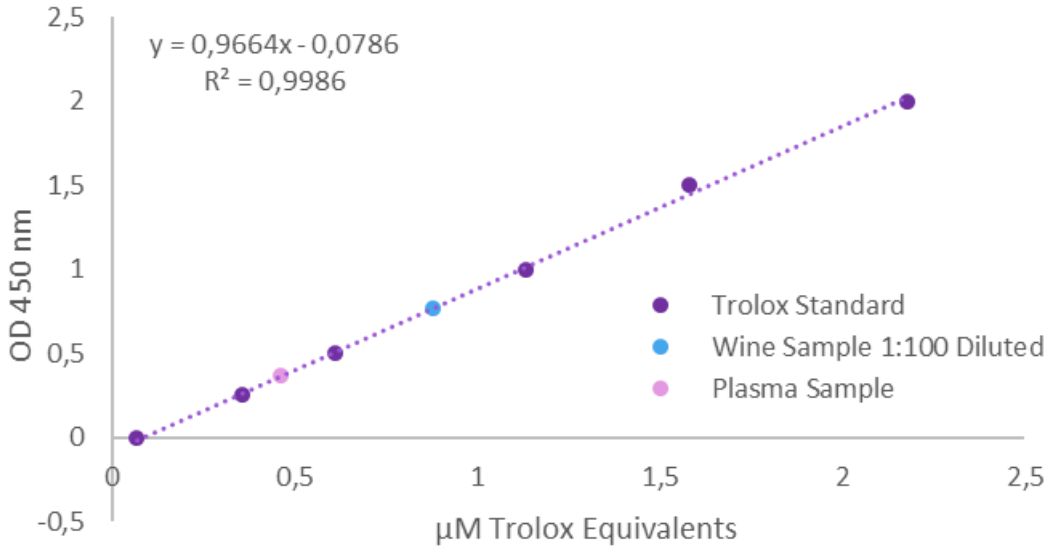


**Figure 3. Example of a typical standard curve. This is just an illustrative example, do not use this graphic for your calculations.**

## Analysis of the Samples:

Determine the unknown sample concentration using the standard curve from the assayed sample value. If a sample blank was performed, subtract the value for Average the OD for the replicates and then apply:

$$\text{µM Trolox Equivalents} = \left(\frac{OD\ 450\ nm - intercept}{slope}\right) * dilution\ factor$$

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