

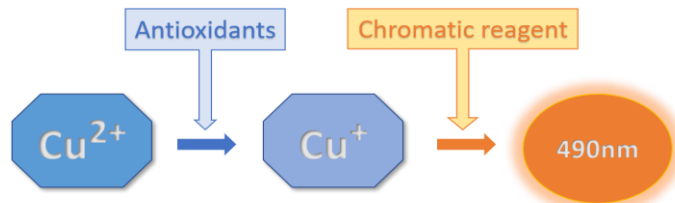
Total antioxidant Capacity (TAC)- CUPRAC Colorimetric Assay (TBS2068-200)

DESCRIPTION

Oxidants, such as reactive oxygen species (ROS) can generate free radicals that can cause severe oxidative damage to cellular lipids, membranes, proteins, and DNA. Antioxidants can scavenge these free radicals and prevent cellular oxidative stress. Antioxidants are widely used as dietary supplements and in industry as preservatives in food, and cosmetics. Measurements of the total antioxidant capacity (TAC) are used an integrated index to assess their ability to counteract oxidative stress-induced damage in cells.

TribioScience’s Total Antioxidant Capacity Colorimetric Assay provides a quick, efficient, and sensitive method to measure TAC from cell lysate, plasma serum, urine, tissue homogenates, and food extracts. This assay is based on the reduction of Cu²⁺ to Cu⁺ by antioxidants like Trolox (Fig1). The resulting Cu⁺ specifically forms a colored complex with a colorimetric probe. The color intensity at 490nm is proportional to TAC in the sample.

Fig. 1 The Reaction Principle



APPLICATION

- **Direct Assays:** serum, plasma, urine, tissues and other biological samples, food and beverages.
- **Drug Discovery/Pharmacology:** Effects of drugs on TAC.

FEATURES

- Sensitive and accurate: Sensitive and accurate. Use 20 μL sample. Linear detection range from 15.63 to 1000 μM Trolox equivalents.
- Simple and high throughput: The procedure involves addition of a single working reagent and incubation for 10 min. Can be readily automated as a high-throughput assay for thousands of samples per day.

KIT CONTENTS FOR 200 ASSAYS

Component	200 Assays	Part Number
Cu ²⁺ Solution	10 mL	TBS2068A
Assay Buffer	10 mL	TBS2068B
Probe	20 mL	TBS2068C
Trolox Standard (1mM)	0.5 mL	TBS2068D

Storage conditions: Store the kit at -20°C, protected from light. Shelf life: 6 months.

ASSAY PROCEDURES

Sample Preparation

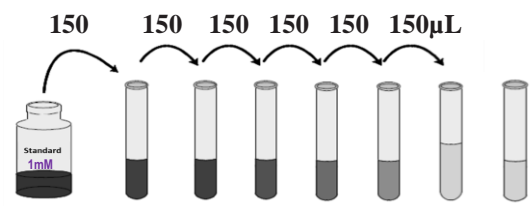
The kit has been tested with serum, urine, culture media, food and drinks. No sample purification from these sources is necessary. If only small molecule TAC is desired, samples should be diluted 1:1 with Assay Buffer. Sample volumes between 0 -20 μl can be assayed per well and should be done in duplicate. For serum samples, we suggest assaying 0.01 - 0.1 μl without Assay Buffer, or 1 - 10 μl with Assay Buffer. All well volumes should be adjusted to 100 μl with ddH₂O.

The absorbance of samples should be in the linear range of the standard curve (0 – 1000μmol). If they fall outside of this range, they should be re-diluted and rerun. The detection limit of the assay is approximately 10 μM of Trolox.

Standard Curve Preparations:

- 2.1 Label 1.5mL Std tubes 2-8. As shown below in the diagram Fig.2.
- 2.2 Add 150 μL of 1x Assay Buffer to Std 2-8.
- 2.3 Carry out a 2x serial dilution for Std 2-7 by adding 150 μL from last concentration to the next concentration. Leave Std8 in 1x Assay Buffer as 0 standard. The standard concentration range is 1000, 500, 250, 125, 62.5, 31.25, 15.63 μM, and 0.

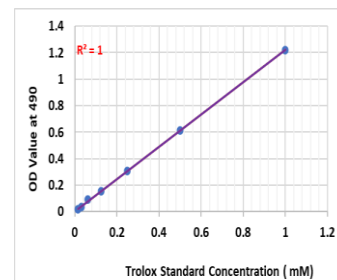
Fig. 2 Diagram for standard preparation



	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Std8
Buffer (μL)	0	150	150	150	150	150	150	150
Addition	Std1	Std2	Std3	Std4	Std5	Std6		
Add (μL)	500	150	150	150	150	150	150	
Final Conc.(μM)	1000	500	250	125	62.5	31.25	15.63	0

Assay Procedures

1. Pipet 20 μL of standard, samples, controls, into individual wells of a microplate.
2. Add 50 μL Cu²⁺ Solution to each well.
3. Add 80 μL of Probe to each well.
4. Incubate at RT for 10 min.
5. Measure OD at 490nm
6. Correct absorbance from background. For each point, subtract the value derived from the control.



RELATED PRODUCTS:

- Amplex Red Hydrogen Peroxide Assay Kit (TBS2066)
- AmplexRed HRP-System (TBS5026)
- Tryptase Activity Assay (TBS2101)
- β-Hexosaminidase Activity Assay (TBS2105)
- Cytochrome C Oxidase Activity Assay (TBS2115)
- Fast Glucose Determination Colorimetric/Fluorometric Assay (TBS2087)
- Glucose Oxidase Activity Colorimetric/Fluorometric Assay (TBS2088)
- Non-esterified Fatty Acid Assay (TBS2203)
- Glycerol Colorimetric / Fluorometric Assay (TBS2204)
- Protein Assay Kits (TBS2005)
- Cell Nuclear Extract kit (TBS6025)

For research use only.