TAC-BCS Antioxidant Assay Kit

Cat# AOX-5

INSTRUCTION MANUAL ZBM0089.00

STORAGE CONDITIONS

All orders are delivered via Federal Express Priority courier at 4°C.
All orders must be processed immediately upon arrival.

Cu(II)SO_4 and Stop Solution
Store at room temperature

Assay Buffer
Store at 4°C.

Trolox and Uric acid standards
Store at -20°C

Long-term storage:

Remove the Assay Buffer from the box and place at 4°C, store the Trolox and Uric acid standard solutions at -20°C. Reagents are good for at least 3 months after arrival if stored properly.

For *in vitro* Use Only

LIMITED PRODUCT WARRANTY

This warranty limits our liability to replacement of this product. No other warranties of any kind, expressed or implied, including without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by Zen-Bio, Inc. Zen-Bio, Inc. shall have no liability for any direct, indirect, consequential, or incidental damages arising out of the use, the results of use, or the inability to use this product.

ORDERING INFORMATION AND TECHNICAL SERVICES

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INTRODUCTION

Free radicals and reactive oxygen species (ROS) are highly reactive molecules that are generated by normal cellular processes, environmental stresses, and UV irradiation. ROS react with cellular components, damaging DNA, carbohydrates, proteins, and lipids causing cellular and tissue injury. Excess production of reactive oxygen species can also lead to inflammation, premature aging disorders, and several disease states, including cancer, diabetes, and atherosclerosis. Organisms have developed complex antioxidant systems to protect themselves from oxidative stress; however, excess ROS can overwhelm the systems and cause severe damage.

The Zen-Bio TAC-BCS Antioxidant Assay Kit can be used to determine the total antioxidant capacity of biological fluids, cells, and tissue. It can also be used to assay the antioxidant activity of naturally occurring or synthetic compounds for use as dietary supplements, topical protection, and therapeutics. The assay measures the reduction of copper(II) to copper(I) in the presence of the aromatic chelator, bathocuproinedisulfonate (BCS). Uric acid or Trolox [6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid], a water soluble vitamin E analog, serves as a positive control for the reduction reaction in a dose dependent manner. The antioxidant activity in biological fluids, cells, tissues, and natural extracts can be normalized to equivalent Uric acid or Trolox units to quantify the composite antioxidant activity present.

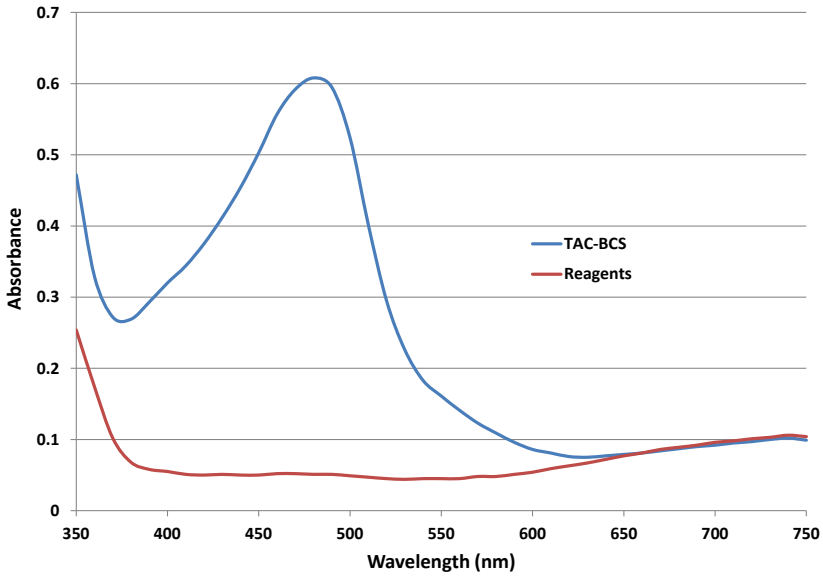
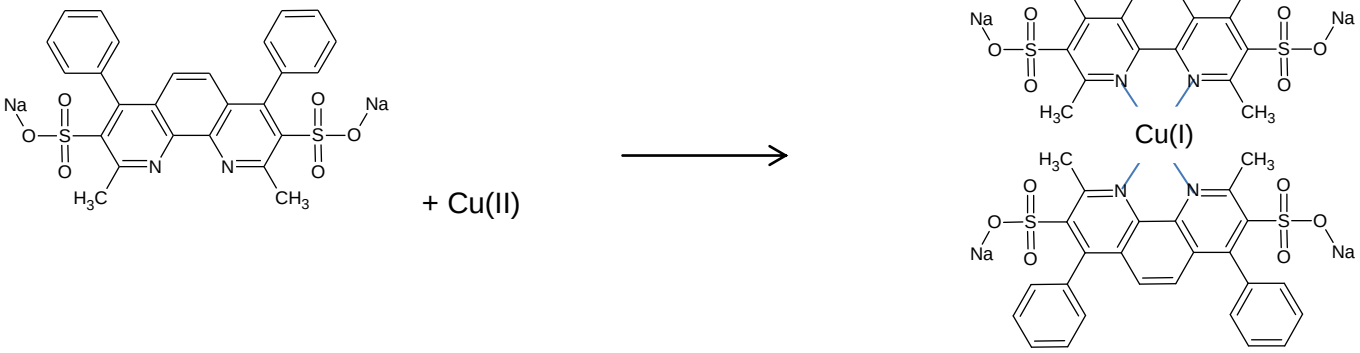


Figure 1. Cu(I) bathocuproine (Cu(I)BCS) complex absorbance spectrum

PRINCIPLE OF THE ASSAY

Copper (II) can be reduced to copper (I) by antioxidants, such as trolox or uric acid, through an electron transfer mechanism. The Cu(I) is stabilized by the bathocuproinedisulfonate in a complex that blocks its oxidation back to Cu(II). ThisCu(I)-BCS complex produces a color that has an absorbance maximum at 490 nm. By comparing the 490 nm absorbance induced by test samples to that induced by the trolox or uric acid standard curve, the relative antioxidant activity of test samples can be determined.



ITEMS INCLUDED IN THE KIT

ITEM	DESCRIPTION	Color	UNIT	QTY	STORAGE
Blank Assay Plate	96-well assay plates, blank	---	PLATE	1	-----
Cu(II)SO_4 solution	6 ml	CLEAR	BOTTLE	1	RT
Assay Buffer	30 ml	CLEAR	BOTTLE	1	4°C
Stop Solution	6 ml	CLEAR	BOTTLE	1	RT
AOX5 Trolox	5mM	AMBER	50 µl /VIAL	1	-20°C
AOX5 Uric Acid	1mM	AMBER	250 µl /VIAL	1	-20°C
Tray	For multi-channel pipetters	---	EACH	3	RT

Other equipment/reagents required but not provided with the kit:

- Multi-channel Pipet , single channel pipet and pipet tips
- Water
- Plate reader with a filter of 490 nm, or within a range of 450 – 510 nm (See figure 1).

Reagents that might interfere with the assay results:

EDTA	EGTA and other chelators
> 1% TWEEN 20	2-mercaptoethanol
> 1% TRITON X-100	Tris
IGEPAL CA-630 (Nonidet P-40)	Borate
> 0.2% CHAPS	DTT

SAMPLE PREPARATION

Cell Lysate Preparation

1. Scrape ~1 x10^6 cells and centrifuge at 1,000xg to prepare a cell pellet. DO NOT use proteolytic enzymes such as trypsin but scrape using a rubber policeman or cell scraper tool.
2. Homogenize or sonicate the cell pellet on ice in 1ml cold PBS
3. Centrifuge at 10,000 x g for 15 minutes at 4°C.
4. Remove the supernatant and keep on ice until ready to use in the assay.
5. If not using the same day, store the samples at -80°C.
6. Data is expressed as Trolox or Uric Acid equivalents (TE or UAE) per cell number (i.e. µmole TE/10^6 cells)

Tissue Lysate Preparation

1. Homogenize tissue samples on ice in cold PBS at ~200mg tissue per ml cold buffer
2. Centrifuge at 10,000 x g for 15 minutes at 4°C.
3. Remove the supernatant and keep on ice until ready to use in the assay.
4. If not using the same day, store the samples in small aliquots at -80°C.
5. Data is expressed as Trolox or Uric Acid equivalents (TE or UAE) per gram of starting sample (i.e. µM TE/g)

Plasma Preparation

1. Collect the blood in a tube containing heparin **[NOT EDTA]**.
2. Centrifuge at 1,000 x g for 10 minutes at 4°C.
3. Remove the supernatant and keep on ice until ready to use in the assay.
4. If not using the same day, store the samples in small aliquots at -80°C.
5. Data is expressed as micromoles Trolox or Uric Acid equivalents (TE or UAE) per volume sample (i.e. µmole TE/L)

Serum Preparation

1. Collect the blood in a tube WITHOUT any anticoagulant. Allow the blood to clot.
2. Centrifuge at 2,000 x g for 10 minutes at 4°C.
3. Remove the supernatant and keep on ice until ready to use in the assay.
4. If not using the same day, store the samples in small aliquots at -80°C.
5. Data is expressed as micromoles Trolox or Uric Acid equivalents (TE or UAE) per volume sample (i.e. µmole TE/L)

Urine Preparation

1. Collect urine and test undiluted or diluted with PBS.

Food Extract Preparation

Weigh the starting material.

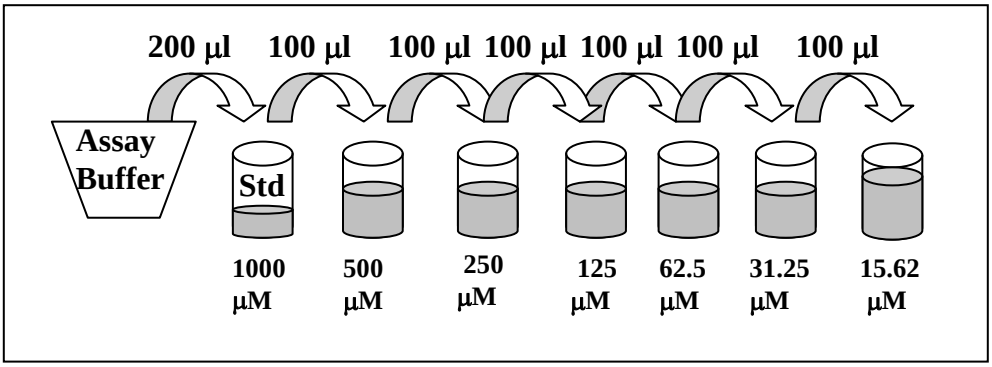
1. Homogenize in a small volume ice cold PBS or water.
2. Store small aliquots at -80°C for analysis.
3. When ready to assay, keep thawed samples on ice.
4. Data is expressed as Trolox or Uric Acid equivalents (TE or UAE) per gram of starting sample (i.e. µM TE/g)

ASSAY PROCEDURE

1. Remove reagents from the refrigerator and allow them to come to room temperature.

2. Prepare standards as follows (either Trolox or Uric acid may be used):

   Trolox: Briefly spin down the contents of the 5 mM Trolox standard tube after thawing. Pipette 200 µl of Assay Buffer into the 5 mM Trolox standard tube provided and mix well by vortexing. This produces a diluted stock Trolox standard of 1000 µM. Pipette 100 µl of Assay Buffer into 6 tubes (not provided). Using the newly diluted stock Trolox solution, prepare a dilution series as depicted below. Mix each new dilution thoroughly before proceeding to the next. The 1000 µM stock dilution serves as the highest standard, and Assay Buffer serves as the zero standard.



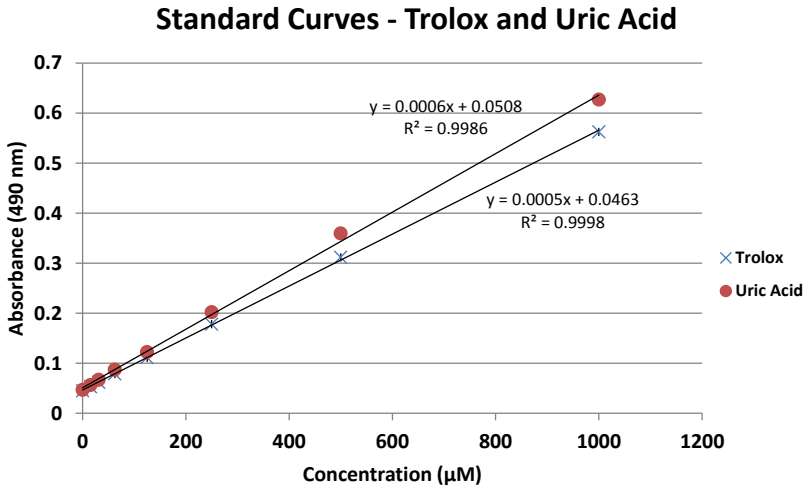
   Uric Acid: Briefly spin down the contents of the 1000 µM Uric Acid standard tube after thawing. Pipette 100 µl of Assay Buffer into 6 tubes (not provided). Using the stock 1000 µM Uric acid solution, prepare a dilution series as depicted above. Mix each new dilution thoroughly before proceeding to the next. The 1000 µM stock dilution serves as the highest standard, and Assay Buffer serves as the zero standard.

3. Add 190 µl of Assay Buffer to each well.
4. Add 10 µl of the diluted standards or samples to the appropriate wells.
5. Read absorbance at 490 nm to determine intrinsic absorbance of your samples.
6. To begin the assay, add 50 µl of the $Cu(II)SO_4$ solution per well and incubate for 5 minutes.
7. Add 50 µl of Stop Solution to each well.
8. Read absorbance using plate reader at a wavelength of 490 nm.

STANDARD CURVE

Generate standard curve: see example below
[DO NOT use this standard curve to generate your data. This is an example.]

µM Trolox	Avg. Abs.	Std Dev.
1000	0.563	0.0290
500	0.312	0.0007
250	0.178	0.0057
125	0.111	0.0000
62.5	0.079	0.0007
31.25	0.062	0.0007
15.625	0.053	0.0000
0	0.045	0.0000



y = observed O.D.
x = concentration of Trolox in µM
To calculate x for each y, (i.e. to change the observed O.D. into Trolox equivalent concentration) use the following equation:
y=(slope) times (x) plus intercept
y=mx+b so x=(y-b)/m
x=(y – 0.0463)/(0.0005) where (0.0005)= slope of the line and 0.0463= y intercept. Be careful to enter the proper sign for the y intercept value as it may be a negative number.

The R^2 value should be equal or greater than 0.98 for the standard curve to be valid. Any R^2 values below 0.98, must have the standard curve run again.

Antioxidant activity is expressed as µM Trolox equivalents (TE) or Uric acid equivalents (UAE) if it was used as the standard.

APPENDIX A: Plate layout _____

	1	2	3	4	5	6	7	8	9	10	11	12
A	Standard 1000 µM	Standard 1000 µM										
B	Standard 500 µM	Standard 500 µM										
C	Standard 250 µM	Standard 250 µM										
D	Standard 125 µM	Standard 125 µM										
E	Standard 62.5 µM	Standard 62.5 µM										
F	Standard 31.25 µM	Standard 31.25 µM										
G	Standard 15.62 µM	Standard 15.62 µM										
H	Standard 0 µM	Standard 0 µM										

APPENDIX B: Protocol Flowchart

TAC-BCS ASSAY

Make necessary test compound dilutions in water or assay buffer.

↓

Prior to assay, allow all reagents to equilibrate to room temperature, prepare standards.

↓

Add 190 µl/well Assay Buffer to blank assay plate.

↓

Add 10 µl/well standards or samples to appropriate wells.

↓

Read absorbance at 490 nm and record intrinsic compound absorbance.

↓

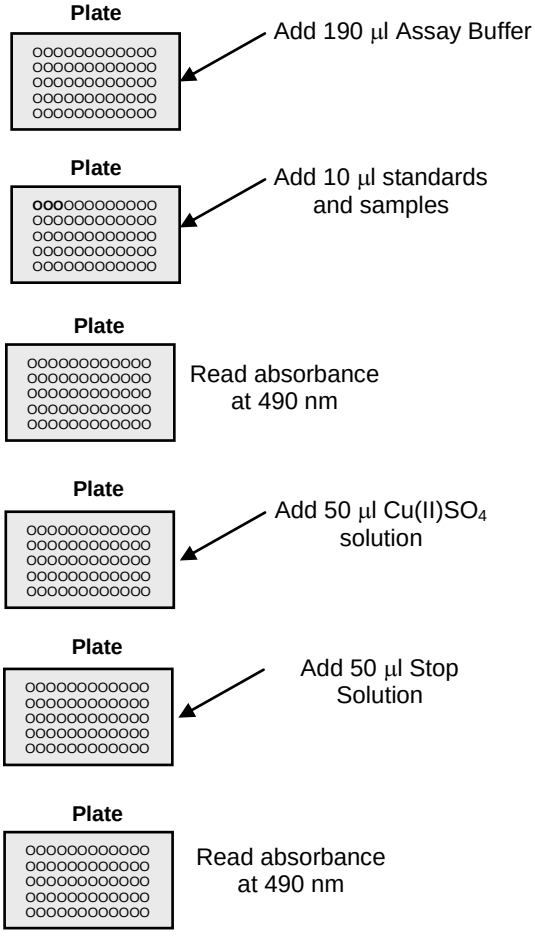
Start assay by adding 50 µl/well Cu(II)SO_4 solution.

↓

Incubate at room temperature for 5 minutes.

↓

Read absorbance at 490nm



ZenBio TAC-BCS and Cu-TAC assays:
Both assays monitor the reduction of Cu(II) to Cu(I) which is stabilized by an aromatic chelator. The TAC-BCS assay is rapid and works quite well with aqueous antioxidants and water soluble components of biological fluids and extracts. The Cu-TAC reaction is slower and requires higher temperature; however, it is capable of assessing lipophilic antioxidants in plasma, tissue homogenates and other extracts.

REFERENCES

1. Analytical Biochem., 392: 37-44, 2009.
2. Analytical Biochem., 423: 36-38, 2012.