MA-0106

Total Antioxidant Capacity (TAC) Assay

(100 wells, Absorbance-based, OD 570 nm, Store at 4°C)

Background Information:

Antioxidants function to minimize damage to primarily proteins, lipids, and DNA caused by reactive oxygen species (ROS) and/or reactive nitrogen oxide species (RNOS). There are three basic categories of antioxidants: enzymes (GSH reductase, catalase, peroxidase, etc.), other nonenzymatic proteins (albumin, transferrin, etc.) and small molecules (ascorbate, uric acid, GSH, vitamin E, etc.). There are several orders of magnitude difference in the reducing power of different. Trolox, a water-soluble vitamin E analog is used as a standard to evaluate the effectiveness of other antioxidants. Measurement of the total antioxidant capacity of biological samples provides an indication of the effectiveness in counteracting reactive oxygen and nitrogen species. It is sometimes necessary to evaluate the contribution of proteins separately from small molecule antioxidants. AkrivisBio's TAC Assay is designed to measure the combination of small molecule antioxidants and proteins, either combined or together. In the TAC Assay, Cu^{2+} ion is converted to Cu^{1+} by all available reducing species. The Protein Mask prevents Cu^{2+} reduction by protein, enabling the analysis of the contribution of only the small molecule antioxidants. The reduced Cu^{1+} ion is subsequently bound to a chromogenic ligand giving a broad peak with λmax at 570 nm, proportional to the total antioxidant capacity.

Assay Principle:

1 – Sample reduces added Cu^{2+}. If protein contribution is to be prevented, protein mask is added.
2 – Chromogenic ligand binds Cu^{1+} resulting in absorbance band at 570 nm.

Assay Components:

Cu^{2+} Reagent	0.2 ml	Blue	MA-0106A
Assay Diluent	10 ml	WM	MA-0106B
Protein Mask	10 ml	NM	MA-0106C
Trolox Standard	1 µmol	Yellow	MA-0106D

Reagent Preparation:

Cu^{2+} Reagent, Assay Diluent, Protein Mask: Ready to use as supplied.

Trolox Standard: Dissolve Trolox in 100 µl DMSO. Take 10 µl (100 nmoles) as needed (enough for 1 std curve), dilute with 240 µl of DI H_2O and mix well. This is a 0.4 mM standard solution.

Preparation of working solution: Take 2 µl of Cu^{2+} reagent + 98 µl Assay diluent for each well to be assayed. Prepare sufficient working solution for the number of wells to be run.

Assay Protocol:

1. Trolox standard curve: Add 0 - 10 - 20 – 30 – 40 - 50 µl of the Trolox standard to a series of wells in a 96-well plate. Adjust the well volume to 100 µl with DI H_2O to give 0, 4, 8, 12, 16, 20 nmol of Trolox standard.

2. Preparation of sample: The kit has been tested with a variety of sample types. No sample treatment is necessary. If the reducing power due to protein is to be suppressed, dilute samples with an equal volume of the Protein Masking Reagent. Use sample volumes of 0 - 100 µl per well, preferably in duplicate. Adjust all wells to 100 µl with DI H_2O.

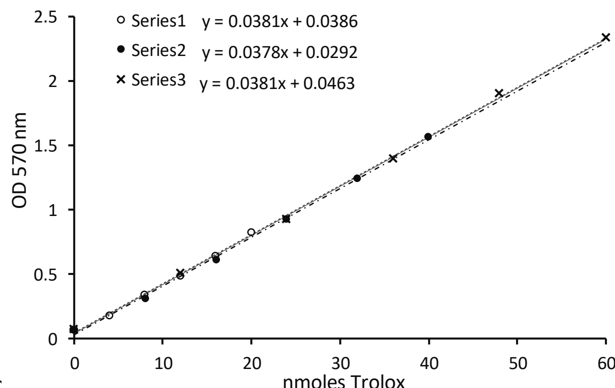
Note: The assay is linear to at least 2.5 OD. The standard curve only goes to about 0.9 OD. If the absorbance of any sample is outside the range of the standard curve, in general it does not need to be diluted.

3. Assay procedure:

A. Add 100 µl Cu^{2+} working solution to all standard and sample wells.
B. Cover the plate and allow color development to proceed at RT for 1.5 hours. Read the absorbance at 570 nm using a plate reader.

4. Typical Result:

nmoles Standard	Standard Raw Values 1	2	3	OD Background Corrected Values 1	2	3
0	0.0657	0.0648	0.0693	0	0	0
4	0.1712			0.1055		
8	0.3373	0.3114		0.2716	0.2466	
12	0.4807		0.5051	0.4150		0.4358
16	0.6395	0.6121		0.5738	0.5473	
20	0.8225					
24		0.9229	0.9243		0.8581	0.8550
32		1.2418			1.1770	
36			1.3972			1.3279
40		1.5636			1.4988	
48			1.9011			1.8318
60			2.3366			2.2673



The lower detection limit of the assay is approximately 0.1 nmol per well (c

5. Calculations

Subtract the 0 µg Trolox Standard OD from all readings. Plot the Standard Curve. Determine the slope of the Standard Curve. The slope defines the OD/µg. Divide the background corrected sample readings by the slope of the Standard Curve to determine the sample Trolox equivalent (TE) in the well. To extrapolate back to the Trolox equivalent in the original samples:

A. Divide the obtained value by the volume of sample added to the well = TE/ µl sample
B. Multiply the TE/ µl sample by the total volume of the sample = total TE / sample
C. If the sample is an extract of cells or tissue, divide the total TE/sample by the mg tissue or # of cells used = TE / mg tissue (# of cells, etc.)

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