Kushan Zist

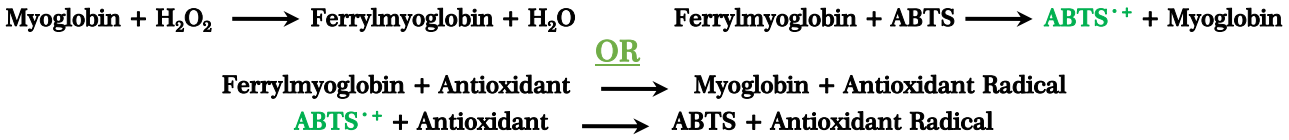
Instruction manual for:

Total Antioxidant Capacity (TAC) Assay Kit

1 About This Kit

Total antioxidant capacity (TAC) is a common term that refers to the assessment of the antioxidant status of a biological system and evaluates the antioxidant response against free radicals produced in that particular system. In living organisms, antioxidants act as a cooperative network, employing a series of redox reactions carried out by macro- and micro-molecules and enzymes. Therefore, the total antioxidant capacity of a biological system provides more relevant biological information compared to the measurement of individual components.

This product measures TAC in biological samples by utilizing a peroxidase chromogenic substrate (ABTS) that produces a water-soluble (blue-green) chromogen upon oxidation by Ferryl Myoglobin radicals. The rate of formation of colored chromogen is inhibited by the presence of antioxidants and can be measured photometrically.



Kit Components

Item Label	Amount	
	48 Tests Kit	96 Tests Kit
10X Assay Buffer	7 ml	12 ml
Chromogen Substrate	Lyophilized	Lyophilized (2 vials)
Myoglobin	Lyophilized	Lyophilized (2 vials)
TROLOX Standard	Lyophilized	Lyophilized
Hydrogen Peroxide	150 µl	250 µl
STOP Solution	4 ml	8 ml
96 Well Microplate	1 Plate	1 Plate
Instruction manual	1 Manual	1 Manual

Storage and Stability

- This kit will perform as specified if **stored at 4°C**.
- Use before the **expiration date** indicated on the box.

Kit Performance

- **Precision:** Human TAC with 10 replicates on 3 different days showed the intra and inter assay coefficient of variation 4.1% and 4.7% respectively.
- **Assay range:** 45 – 420 µM
- **Application:** For *in vitro* measurement of TAC in serum, plasma, tissue, cell lysates and other biological fluids.

Materials Required (Not Provided)

- Double distilled water (ddH_2O)
- Microtubes, 15 ml / 50 ml tubes
- Dounce homogenizer (if using tissue samples)
- Sonicator (if using cell lysate)

Contact us on:

Sale: +98 21 66381732 **Email:** sale@kushanzist.com **Technical:** +98 9913399155
+98 921 020 5601 **Web:** http://www.kushanzist.com **Hours:** S-T 8:30 – 16:00

Required Instrumentation

- Multiwell microplate reader capable of measuring absorbance at 412±7 nm.
- Microfuge
- Pipettes with variable volume setting.

Warning and Precautions

- It is recommended that gloves, lab coat, and protective eyewear be worn at all times.
- In case of contact with skin or eyes, wash thoroughly with soap and cold water.
- Consider all components as hazardous and dispose according to established safety laws.

Sample Preparation

Note: Assay all samples immediately upon extraction or store in working aliquots at -80°C for up to 1-2 months.

Note: Thaw samples on ice (Regulation of temperature at 4°C increases sensitivity and reproducibility).

Note: Use 1X Assay buffer for extraction or dilution purposes as reagents present in different buffers may interfere with the assay.

Note: Before your formal assay, perform a pre-assay by preparing a dilution series for 2-3 samples and determine a dilution factor that will bring the antioxidant levels of the samples within the range of the Standard Curve.

PLASMA

1. Collect whole blood using an anticoagulant such as heparin or citrate. Do not use EDTA.
2. Centrifuge at 1,000 x g for 10 minutes at 4°C.
3. Carefully pipette off the plasma layer without disturbing the buffy layer, transfer to fresh tubes and place on ice.

SERUM

1. Collect whole blood without using an anticoagulant.
2. Allow blood to clot for 30 minutes at 25°C.
3. Centrifuge the blood at 2000 x g for 15 minutes at 4°C and
4. Carefully pipette off the yellow serum layer without disturbing the buffy layer and transfer to new tubes and place on ice.

TISSUE HOMOGENATES

1. Weight out the amount of tissue necessary for each assay (~100 mg wet tissue).
2. Homogenize the tissue in 0.5 ml ice cold 1X Assay Buffer.
3. Centrifuge at 10,000 x g for 15 minutes at 4°C.
4. Collect the supernatant and transfer to fresh tubes and place on ice.

CELL LYSATES

1. Harvest the number of cells necessary for each assay (~1 x 10^6 cells/ml recommended). For harvesting adherent cells, don't use proteolytic enzymes (like trypsin); rather use a rubber policeman.
2. Lyse cells by repeated freeze/thaw cycles or sonication in ice cold 1X Assay Buffer.
3. Centrifuge at 10,000 x g for 15 minutes at 4°C.
4. Collect the supernatant and transfer to fresh tubes and place on ice.

URINE

1. Collect urine in a clean container and place on ice.

SALIVA

3

1. Collect saliva in a clean container and place on ice

Reagent Preparation

1) **1X Assay Buffer:** Dilute 10X Assay Buffer ten-fold with ddH_2O (e.g., add 3 ml of 10X Assay Buffer to 27 ml of ddH_2O) and mix well. 1X Assay Buffer is stable for six months at 4°C.
2) **Chromogen Solution:** Spin the Chromogen Substrate vial for 30 seconds. Add 1 ml of 1X Assay Buffer to the vial and vortex. Transfer the contents of the vial to a fresh tube containing 5 ml of 1X Assay Buffer, vortex well and protect from light. One reconstituted vial is sufficient for 48 wells. Chromogen Solution is stable only for 24 hours at 4°C.
3) **Myoglobin Solution:** Spin the Myoglobin vial for 30 seconds. Add 1.6 ml of 1X Assay Buffer to the vial, vortex well and protect from light. The Myoglobin Solution remains active for a month at –20 °C. One reconstituted vial is sufficient for 48 wells.
4) **Standard Stock Solution:** Spin the TROLOX Standard vial for 30 seconds. Add 2.65 ml of 1X Assay Buffer to the vial and vortex well until totally dissolved. This reconstituted 1.5 mM Standard Stock Solution is used to prepare the standard curve. Standard Stock Solution is stable for 3 months at –20 °C.
5) **Hydrogen Peroxide Solution:** The Hydrogen Peroxide vial contains an ~0.9 M solution of H_2O_2. Dilute 10 µL of this solution with 9 ml of ddH_2O to prepare ready Hydrogen Peroxide Solution. This reagent is stable for 4 hours at room temperature.

STANDARD PREPRATION

- Take six clean microtubes and label them A-F.
- Add the amount of Standard Stock Solution and 1X Assay Buffer to each tube as below.

Tubes	Standard Stock Solution (µl)	1X Assay Buffer (µl)	Final Concentration (µM)
A	0	500	0
B	5	495	15
C	15	485	45
D	35	465	105
E	70	430	210
F	140	360	420

Things to Note

- Allow all reagents to equilibrate to room temperature prior to assay.
- Assay all samples and standards in duplicate/triplicate.
- Use multichannel pipettes to avoid reaction time lag of each well.
- The final volume in each well is 200 µl.
- Use 1X Assay Buffer to dilute samples.
- The TAC inhibition ratio can reach 100%. If so, dilute and re-assay.
- Pay proper attention to your pipetting technique to avoid erratic results in sample replicates.

Assay Protocol

1. Shake all samples to homogenize.
2. Add 10 µl of Standards/Samples to related wells.
3. Add 100 µl of Chromogen Solution to all wells.
4. Add 20 µl of Myoglobin Solution to all wells and incubate for 5 minutes.
5. Add 20 µl Hydrogen Peroxide Solution to all wells to initiate the reaction. Perform this step as quickly as possible (*multichannel pipettes are preferred at this step*).
6. Light-protect the plate and incubate on shaker at room temperature for 5 minutes.

Note: The five-minutes incubation is suggested as a guideline. If required, the incubation time can be changed (increased or decreased) to obtain a measurable absorbance.

7. Add 50 µl of Stop Solution to all wells.
8. Using a plate reader, read the absorbance at 412±7 nm within one hour.

Calculations

1. Calculate the average absorbance of the wells for each standard and sample.
2. Plot the average absorbance values of each standard as a function of the final standard concentration (µM).
3. Calculate the antioxidant concentration for each sample using the equation obtained from the standard curve.

$$\text{TAC } (\mu\text{M}) = \frac{(Sample\ average\ absorbance) - (Intercept)}{(Slope)} \times dilution\ factor$$

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Absorbance <0.05 in sample wells	High concentration of antioxidants in samples	Dilute samples with 1X Assay Buffer and re-assay
	Interference reagent in samples	
No antioxidants were detected in samples	Sample was too dilute	Re-assay with lower dilutions
Erratic values; dispersion of duplicate/triplicates	Poor pipetting/technique	Be careful not to splash the contents of the wells
	Bubble in wells	Remove bubbles by tapping

Contact us on:

Sale: +98 21 66381732 **Email:** sale@kushanzist.com **Technical:** +98 9913399155
+98 921 020 5601 **Web:** http://www.kushanzist.com **Hours:** S-T 8:30 – 16:00