Technical Service Contact Information
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Hours: S-T 8:00 AM to 3:30 PM

TEB PAZHOUHAN RAZI

(TPR)

Total Antioxidant Capacity (TAC) Assay Kit

(48/96 Tests)

Storage and Stability
This kit will perform as specified if **stored at 4°C**
Use before the **expiration date**

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About This Kit

Reactive oxygen species (ROS) are produced as a consequence of normal aerobic cell metabolism and as a response to exogenous factors including UV light, cigarette smoke, environmental pollutants, etc. Once formed, ROS easily react with other molecules within cells and cause damage to cellular and intracellular structures, including cell membranes, proteins, DNA and mitochondria. ROS are known to be one of the main causative factors involved in the pathogenesis of diverse diseases such as neurodegeneration, diabetes, cancer, rheumatoid arthritis, atherosclerosis, etc. Physiologically, there are complicated systems of defense against ROS-induced tissues damage which provided by macro and micro molecules and enzymes. Antioxidants act as a cooperative network, employing a series of redox reactions. Thus, quantitative measurement of the total antioxidant capacity (TAC) may provide more relevant biological information compared to that obtained by measurement of individual components.

TPR's TAC Assay Kit is a well-established tool for measuring TAC in serum, plasma, tissues, cell lysates and other biological fluids. This kit utilizes a peroxidase chromogenic substrate, which produces a water-soluble chromogen upon oxidation by ferryl myoglobin radicals. The green chromogen formation rate is inhibited by presence of antioxidants in environments and is measurable photometrically.

Performance

- **Precision:** Human TAC with 10 replicates on 3 different days showed the intra and inter assay coefficient of variation 5.7% and 3.7% respectively.
- **Range:** Samples containing antioxidants levels between 45-420 µM can be assayed without dilution.

Components

Item Label	Item	48 Tests Kit	96 Tests Kit
Reagent 1 (**R1**)	TAC Assay Buffer (10X)	5 ml	5 ml
Reagent 1 (**R2**)	TAC Assay Chromogen	Lyophilized	Lyophilized (2 vials)
Reagent 2 (**R3**)	TAC Assay Myoglobin	Lyophilized	Lyophilized
Reagent 4 (**R4**)	TAC Assay Standard	Lyophilized	Lyophilized
Reagent 5 (**R5**)	TAC Assay Hydrogen Peroxide (14.7 M)	200 µl	200 µl
-	96 Well Microplate	1 Plate	1 Plate
-	Technical Manual	1 Manual	1 Manual

Storage and stability

- This kit will perform as specified if store as directed at 2-8°C.
- Use before the expiration date.

Required Materials Not Provided

- HPLC-grade water.
- Disposables 15 ml and 1.7 ml tubes.
- Dounce homogenizer (if using tissue).
- Sonicator.

Required Instrumentation

- Multiwell microplate reader (capable of measuring absorbance at 412±7 nm or 734±16 nm).
- Microfuge
- 2-20 µl and 20-200 µl pipettes.

Sample Preparation

- **Important Notes:**
  - ✓ Reagents present in different extraction buffers may interfere with the assay, use 1X Assay buffer for extraction instead.
  - ✓ All samples should be assayed immediately upon extraction or stored at -80°C.
  - ✓ Always thaw samples on ice.

Cell Samples:

1. Harvest the amount 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 cycles of freezing and thawing or sonication in 1 ml ice cold 1X Assay Buffer.
3. Centrifuge at 12,000 x g for 15 minutes at +4°C.
4. Collect the supernatant and transfer to fresh tubes and place on ice. For long-term storage, store in working aliquots at –80 °C.

Tissue Samples:

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 12,000 x g for 15 minutes at +4°C.
4. Collect the supernatant and transfer to fresh tubes and place on ice. For long-term storage, store in working aliquots at –80 °C.

Plasma Samples:

Typically, human plasma has an antioxidant capacity of 0.5-2 mM (2, 4, 6, 7).

1. Collect whole blood using an anticoagulant such as heparin or citrate. Don't 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. For long-term storage, store in working aliquots at –80 °C. The plasma samples will be stable for at least one month.

Note: Plasma should be diluted 20-30 times with 1X Assay Buffer prior to assay TAC.

Serum Samples

Typically, human serum has an antioxidant capacity of 0.5-2 mM (8, 9).

1. Collect whole blood without using an anticoagulant.

2. Allow blood to clot for 20 minutes at 25°C.
3. Centrifuge the blood at 2000 x g for 15 minutes at 4°C.
4. Carefully pipette off the yellow serum layer without disturbing the buffy layer and transfer to a new tubes and place on ice. For long-term storage, store in aliquots at –80 °C. The serum samples will be stable for at least one month.

Note: Serum should be diluted 20-30 times with 1X Assay Buffer prior to assay TAC.

Urine Samples

Typically, human urine has an antioxidant capacity of 0.2-3 mM (9).

Collect urine in a clean container and place on ice. For long-term storage, store in aliquots at –80 °C.
Note: Urine should be diluted 10-20 times with Assay Buffer prior to assay TAC.

Saliva Samples

Typically, human saliva has an antioxidant capacity of 0.3-1 mM (9).

Collect saliva in a clean container and place on ice. For long-term storage, store in aliquots at –80 °C.
Note: Saliva should be diluted 2 times with 1X Assay Buffer prior to assay TAC.

Reagent Preparation

1) **1X Assay Buffer**: Dilute R1 (10X Assay Buffer) ten-fold with HPLC-grade water (e.g., add 2 ml of 10X Assay Buffer to 18 ml of HPLC-grade water) and mix well. This 1X Assay Buffer is stable for six months if stored at 4°C.

2) **R2 Ready Reagent: R2** vial contains lyophilized powder of chromogen. Spin **R2** vial for 30 seconds. Add 1 mL of **1X Assay Buffer** to vials and vortex well. Then transfer contents of vials to a tube already containing 7 mL **1X Assay Buffer** and vortex well. One reconstituted vial is sufficient for 48 wells. **R2 Ready Reagent** is stable for 24 hours at 4°C.

3) **R3 Ready Reagent: R3** vial contains lyophilized powder of Myoglobin. Spin **R3** vial for 30 seconds. Add 600 µL of **1X Assay Buffer** to vial (48 Tests Kit)/Add 1.2 mL of **1X Assay Buffer** to vial (96 Tests kit) and vortex well. Store in working aliquots at –20 °C. The **R3 Ready Reagent** remains active for one month at –20 °C.

4) **R4 Ready Reagent: R4** vial contains lyophilized powder of TAC Standard. Spin **R4** vial for few seconds. Add 1 mL of HPLC-grade water to vial and vortex well. Then transfer content of vial to a new tube already containing 1.67 mL of HPLC-grade water and vortex well until totally dissolved. This reconstituted **Standard stock solution** is used to prepare the standard curve. **Standard stock solution** is stable for 24 hours at 4°C.

5) **R5 Ready Reagent:** This vial contains a 14.7 M solution of hydrogen peroxide. Dilute 6.8 µL of **R5** reagent with 993 µL of HPLC-grade water and mix well to obtain 100 mM concentration of R5 reagent. Further dilute by removing 20 µL and diluting with 3980 µL of HPLC-grade water to prepare **R5 Ready Reagent**. **R5 Ready Reagent** is stable for 4 hours at room temperature.

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Preparation of Standard Curve

Take six clean microtubes and label them A-F. Add the amount of standard stock solution and HPLC-grade water to each tube as described in below.

Tubes	Standard Working Solution (µL)	HPLC-grade water (µL)	Standard Concentration (µM)
A	0	500	0
B	15	485	45
C	25	475	75
D	35	465	105
E	70	430	210
F	140	360	420

Things to Note

- Except samples, allow all reagents to equilibrate to room temperature before performing the assay.
- It is recommended that all samples and standards be assayed at least in duplicate.
- The final volume in each well is 200 µL.

Assay Protocol

1. Shake all samples for homogenation.
2. Add 10 µL standards/samples to related wells.
3. Add 150 µL **R2 Ready Reagent** to all wells.
4. Add 10 µL **R3 Ready Reagent** to all wells and incubate for 5 minutes.
5. Add 30 µL **R5 Ready Reagent** to all wells to initiate the reaction. Perform this step as quickly as possible (within one minute is recommended).
6. Cover the plate with plate cover 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) in order to obtain a measurable absorbance.
7. Read absorbance at 734±16 nm or 412±7 nm using a plate reader.

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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.

$$\text{TAC (µM)} = \frac{(Sample\ average\ absorbance\ ) - (\text{y-intercept})}{Slope}\ \text{X}\ dilution\ factor$$

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Absorbance <0.05 in sample wells	1. High concentration of antioxidants 2. Interference reagent in samples	Dilute samples with 1X assay buffer and re-assay
No antioxidant were detected in samples	Sample was too dilute	Re-assay with lower dilutions
Erratic values; dispersion of duplicate/triplicates	1. Poor pipetting/technique 2. Bubble in wells	1. Be careful not to splash the contents of the wells 2. Remove bubbles by tapping the side of plate

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References

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