Total Antioxidant Capacity (TAC) Assay Kit

Catalog No.: abx294131

Size: 96 tests

Detection Range: 0.049 mmol/L – 2.5 mmol/L

Sensitivity: 0.049 mmol/L

Storage: Store all components at 4°C. Store the TPTZ and Substrate in the dark.

Application: For detection and quantification of Total Antioxidant Capacity in serum, plasma, urine, saliva, tissue homogenates, and cell lysates.

Introduction

Abbexa's Total Antioxidant Capacity Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Total Antioxidant Capacity. Under acidic conditions, antioxidants in samples reduce Fe^{3+}-TPTZ to produce blue-colored Fe^{2+}-TPTZ. The blue-colored product has an absorbance maximum at 593 nm. The intensity of the color is proportional to the Total Antioxidant Capacity, which can then be calculated.

Kit components

1. 96-well microplate
2. Buffer Solution: 20 ml
3. TPTZ Solution: 2 ml
4. Substrate: 2 ml
5. Standard ($FeSO_4 \cdot 7H_2O$): 200 mg
6. Plate sealer: 2

Materials required but not provided

1. Microplate reader (593 nm)
2. Double distilled water
3. Normal saline (0.9 % NaCl)
4. PBS (0.01 M, pH 7.4)
5. Pipette and pipette tips
6. 1.5 ml microcentrifuge tubes
7. Dounce homogenizer
8. Ultrasonic cell disruptor
9. Centrifuge
10. Vortex mixer
11. Incubator

Protocol

A. Preparation of samples and reagents

1. Samples

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum/Plasma:** Samples can be tested directly. Analyse immediately or aliquot and store at -80°C for up to 1 month.
- **Urine:** Collect fresh urine into a sterile container and centrifuge at 10,000 × g at 4°C for 10 minutes. Collect the supernatant for detection and analyse immediately or aliquot and store at -80°C for up to 1 month.
- **Saliva:** Gargle with clear water, then collect the saliva 30 minutes later. Centrifuge at 10,000 × g at 4°C for 10 minutes. Collect the supernatant, keep on ice, and assay immediately.
- **Tissue Homogenates:** Weigh 20 mg of sample and wash in cold PBS (0.01 M, pH 7.4). Homogenize with a Dounce homogenizer in 180 µl PBS (0.01 M, pH 7.4), then centrifuge at 10,000 × g at 4°C for 10 minutes. Collect the supernatant, keep on ice, and assay immediately. The protein content of the supernatant should be determined separately **(abx097193)**.
- **Cell Lysates:** Harvest the required amount of cells (initial recommendation: 10^6 cells) and wash with PBS (0.01 M, pH 7.4). Homogenize the cells in 300-500 µl PBS (0.01 M, pH 7.4) using ultrasonic lysis at 4°C, then centrifuge at 10,000 × g for 10 minutes. Collect the supernatant, keep on ice, and assay immediately. The protein content of the supernatant should be determined separately **(abx097193)**.

Samples should not contain detergents such as SDS, Tween-20, NP-40 and Triton X-100, or reducing agents such as DTT or mercaptoethanol.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with normal saline (0.9 % NaCl) or PBS (0.01 M, pH 7.4), then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
Human serum	1
Human saliva	1
Human urine	1
HepG2 cell lysate	1
5 % Mouse liver tissue homogenate	1
10 % *Epiprumnum aureum* tissue homogenate	1

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.
- Reagents with strong blue color may affect the results and should be avoided.
- High concentrations of Fe^{3+} salts or Fe^{2+} salts may affect the results. For reference, serum/plasma samples are expected to have < 10 µM, which is acceptable.
- Low concentrations of metallic chelating agents will not affect the results.

2. Reagents

- **FRAP Working Solution**: Each well requires 180 µl of FRAP Working Solution. Add 15 µl TPTZ Solution and 15 µl Substrate to 150 µl Buffer Solution, and mix thoroughly. Prepare fresh before use and use within 2 hours.
- **100 mmol/L $FeSO_4$ Solution:** Add 27.8 mg of Standard to 1 ml double distilled water. Prepare fresh and use immediately.
- **Standards:** Dilute 100 mmol/L $FeSO_4$ Solution 10-fold with double distilled water, then prepare standard dilutions as summarized in the following table:

Standard Dilution (mmol/L)	0	0.3	0.6	0.9	1.2	1.8	2.1	2.5
10 mmol/L Standard (µl)	0	15	30	45	60	90	105	125
Double distilled water (µl)	500	485	470	455	440	410	395	375

For the blank, or 0 mg/ml standard, use pure double distilled water. The volume of each standard will be 500 µl.

Note:

- Allow all reagents to equilibrate to room temperature before use.
- Fe^{2+} may easily be oxidized to Fe^{3+}; discard 100 mM $FeSO_4$ Solution if a color change is observed from light green to yellow.

B. Assay Procedure

1. Add 5 µl of standard dilutions to standard wells.
2. Add 5 µl of sample to sample wells.
3. Add 180 µl of FRAP Working Solution to each well.
4. Incubate at 37°C for 3-5 minutes, then measure the OD of each well with a microplate reader at 593 nm.

C. Calculation of Results

Plot the standard curve, using the OD of the standard dilutions (adjusted for the blank) on the y-axis, and their respective concentrations on the x-axis. The curve should form a straight line, described by the formula $y = ax + b$. Based on this curve, the concentration of Total Antioxidant Capacity in each sample well can be derived with the following formulae:

1. Serum, Plasma, Urine, and Saliva samples:

$$\text{Total Antioxidant Capacity (mmol/L)} = \frac{\Delta A_{593} - b}{a} \times f$$

2. Tissue samples:

Total Antioxidant Capacity in tissue samples can be calculated according to total protein concentration (which must be assayed separately)

$$\text{Total Antioxidant Capacity (mmol/g protein)} = \frac{\Delta A_{593} - b}{a} \times \frac{f}{C_{prot}}$$

where:

ΔA_{593}	$OD_{sample} - OD_{blank}$
a	Gradient of the standard curve ($y = \boldsymbol{a}x + b$)
b	Y-intercept of the standard curve ($y = ax + \boldsymbol{b}$)
f	The dilution factor of sample
C_{prot}	Concentration of protein in sample (g protein/L)

Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.