**ab65329 Total Antioxidant Capacity Assay Kit (Colorimetric)**

For the sensitive and accurate measurement of antioxidant proteins and/or small molecules in various biological samples. This product is for research use only and is not intended for diagnostic use.

**For overview, typical data and additional information please visit: www.abcam.com/ab65329**
(use abcam.cn/ab65329 for China, or abcam.co.jp/ab65329 for Japan)

## Materials Supplied and Storage

**Store kit at 4°C in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.**
Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components.
Aliquot components in working volumes before storing at the recommended temperature.
**Δ Note:** Reconstituted components are stable for 4 months.

| Item | Quantity | Storage condition (before prep) | Storage condition (after prep) |
|---|---|---|---|
| Assay Diluent | 10 mL | 4°C | RT |
| $Cu^{2+}$ Reagent | 200 µL | 4°C | RT |
| Protein Mask | 10 mL | 4°C | RT |
| Trolox Standard (1 µmol, lyophilized) | 1 vial | 4°C | -20°C |

## Materials Required, Not Supplied

- Microplate reader capable of measuring absorbance at OD 570 nm
- MilliQ water or other type of double distilled water ($ddH_2O$)
- PBS
- DMSO (anhydrous)
- Pipettes and pipette tips, including multi-channel pipette
- Assorted glassware for the preparation of reagents and buffer solutions
- Tubes for the preparation of reagents and buffer solutions
- 96 well plate with clear flat bottom
- Dounce homogenizer (if using tissue)
- (Optional) – Triton X-100 for sample preparation

## 1. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

**1.1 Assay Diluent:** Ready to use as supplied. Equilibrate to room temperature before use. Store at room temperature.

**1.2 $Cu^{2+}$ Reagent:** Ready to use as supplied. Equilibrate to room temperature before use. Store at room temperature.

**1.3 Protein Mask:** Ready to use as supplied. Equilibrate to room temperature before use. Store at room temperature.

**1.4 Trolox Standard:** Reconstitute in 20 µL of pure DMSO by vortexing vial. Then add 980 µL of $fdH_2O$ and mix to generate a 1 mM Trolox Standard solution. Aliquot standard so that you have enough to perform the desired number of assays. Store at -20°C. Use within 4 months.

## 2. Standard Preparation

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Use 1 mM Trolox Standard solution to prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

| Standard# | Trolox Standard (µL) | $ddH_2O$ (µL) | Final volume standard in well (µL) | End amount Trolox in well (nmol/well) |
|---|---|---|---|---|
| **1** | 0 | 300 | 100 | 0 |
| **2** | 12 | 288 | 100 | 4 |
| **3** | 24 | 276 | 100 | 8 |
| **4** | 36 | 264 | 100 | 12 |
| **5** | 48 | 252 | 100 | 16 |
| **6** | 60 | 240 | 100 | 20 |

Each dilution has enough amount of standard to set up duplicate readings (2 x 100 µL).

## 3. Sample Preparation

We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you snap freeze your samples in liquid nitrogen upon extraction and store them immediately at -80°C. When you are ready to test your samples, thaw them on ice and proceed with the Sample Preparation step. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.

### 3.1 Cell (adherent or suspension) samples:

3.1.1 Harvest the amount of cells necessary for each assay (initial recommendation = 2 x $10^6$ cells).
3.1.2 Wash cells with cold PBS.
3.1.3 Resuspend cells in 100 µL of $ddH_2O$ (0.05% Triton can be used if needed).
3.1.4 Homogenize cells quickly by pipetting up and down a few times.
3.1.5 Incubate cell homogenate for 10 minutes on ice.
3.1.6 Centrifuge sample 2 – 5 minutes at 4°C at top speed in a cold microcentrifuge to remove any insoluble material.
3.1.7 Collect supernatant and transfer to a new tube.
3.1.8 Keep on ice.

### 3.2 Tissue samples:

3.2.1 Harvest the amount of tissue necessary for each assay (initial recommendation = 10 – 100 mg).
3.2.2 Wash tissue in cold PBS.
3.2.3 Resuspend tissue in 500 – 1000 µL of ice cold PBS.
3.2.4 Homogenize tissue with a Dounce homogenizer or pestle sitting on ice, with 10 – 15 passes.
3.2.5 Incubate tissue homogenate for 10 minutes on ice.
3.2.6 Centrifuge sample 2 – 5 minutes at 4°C at top speed in a cold microcentrifuge to remove any insoluble material.
3.2.7 Collect supernatant and transfer to a new tube.
3.2.8 Keep on ice.
**Δ Note**: Sonication can be used for tissue preparation if desired.

**3.3 Plasma, serum, urine and cell culture media samples:**
No sample purification is required; samples can be measured directly.
**Δ Note**: For plasma, heparin is recommended.

**3.4 Food samples:**
3.4.1 Liquid samples: no sample preparation is required.
3.4.2 Solid samples: solid food samples should be homogenized following procedure described in step 3.2
**Δ Note**: We suggest using different volumes of sample to ensure readings are within the standard curve range.

## 4 Assay Procedure

Equilibrate all materials and prepared reagents to room temperature prior to use.
We recommend that you assay all standards, controls and samples in duplicate.
Prepare all reagents, working standards, and samples as directed in the previous sections.

**4.1 $Cu^{2+}$ Working Solution:**
Dilute 1 part of $Cu^{2+}$ Reagent (Step 1.2) with 49 parts of Assay Diluent (Step 1.3) – for example, add 9.8 mL of Assay Diluent to the 200 µL of $Cu^{2+}$ Reagent. Mix well.

**4.2 Set up reaction wells:**
SMALL MOLECULE TAC (Total Antioxidant capacity): If only measuring small molecule total antioxidant capacity, samples should be diluted 1:1 with Protein Mask (Step 1.3) prior addition to the well.
- Standard wells: 100 µL Standard dilutions.
- Sample wells = 1 – 100 µL samples (adjust volume to 100 µL/well with $ddH_2O$).

**Δ Note**: For serum, we suggest to assay 0.01 – 1 µL without Protein Mask, or 1 – 10 µL with Protein Mask.

**4.3 TAC Assay:**
4.3.1 Add 100 µL $Cu^{2+}$ Working Solution to all standard and sample wells.
4.3.2 Mix and incubate plate at room temperature for 90 minutes on an orbital shaker protected from light.
4.3.3 Measure output on a microplate reader at OD 570 nm.

## 5. Calculations

Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.
**5.1** Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.
**5.2** Average the duplicate reading for each standard and sample.
**5.3** Plot the corrected absorbance values for each standard as a function of the final concentration of Trolox.
**5.4** Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).
**5.5** Concentration of Trolox (nmol/µL or mM) in the test samples is calculated as:

$$Sample\ Total\ Antioxidant\ Capacity = \left(\frac{Ts}{Sv}\right) * D$$

Where:
Ts = TAC amount in the sample well calculated from standard curve (nmol).
Sv = sample volume added in the sample wells (µL).
D = sample dilution factor.

## FAQs

**Q. Why do I get different results from the same sample at different times?**
A. This can occur due to 3 main possibilities:
1. Different lots may give different readings. However, when using calibration curve together with the sample testing, the sample concentration should be in the same range.
2. Sample may change during storage, especially Vitamin C.
3. When using the same lot, the same sample reading should be similar, otherwise maybe some experimental error.

**Q. Is the theory of TAC assay kit the same as the DPPH radical method? I am using DPPH reagent (final concentration 250 µM in toluene) and want to know is there any advantage for using your TAC assay kit.**
A. The two assays are different from one another. Our TAC assay uses $Cu^{++}$ to $Cu^{+}$ as a mechanistic tool. It is easy to reduce so some molecules such as uric acid will respond quickly to it. Some of the disadvantages of the DPPH are:
1) This method quantifies DPPH after exposure to sample. The standard curve gives negative results.
2) Reaction kinetics between DPPH and anti-oxidants are not linear. So the antioxidant capacity is rather arbitrary.
3) The time to steady state using DPPH varies with different anti-oxidants. So you may get conflicting relative capacity depending on the reaction time.

**Q. Does EDTA in blood affects the use of this kit on plasma?**
A. Although small presence of EDTA should have no problem on the function of this kit, we recommend using heparin or citrate. Samples that use EDTA as anticoagulant will give a lower antioxidant capacity compared with heparinized plasma samples, but should nonetheless be sufficient for detection and data analysis.

**Q. Is the protein mask necessary with urine sample, since the level of protein in urine compared to serum is minimal?**
A. If the total antioxidant capacity is desired, I would not recommend the use of the protein mask. If only the levels of the small molecule antioxidants are required, please use the protein mask.

**Q. Which sample is better between serum and plasma?**
A. Both human serum and plasma have an antioxidant capacity of 0.5 to 2 mM. So both samples would be equally good for detection.


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