Total Antioxidant Capacity Assay Kit

Iron Assay Kit is a detection kit for the quantification of Total Antioxidant Capacity in urine, serum, plasma, tissue extracts, cell lysate and cell culture supernatants.

Catalog number: ARG82013

Package: 96 wells

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PRINCIPLE OF THE ASSAY

This kit is designed to measure Total Antioxidant Capacity in urine, serum, plasma, tissue extracts, cell lysate and cell culture supernatants. Fe^{3+} in the sample is reduced to Fe^{2+}, and Fe^{2+} was complexed with a chromogen to form a blue to purple colored complex. This assay kit measures total antioxidant capacity in which Fe3+-TPTZ is reduced by antioxidant to Fe2+-TPTZ. The enzyme catalysed reaction products Fe2+-TPTZ can be measured at a colorimetric readout at 593 nm. The Total Antioxidant Capacity in the sample is then determined by comparing the O.D. of samples to the standard.

MATERIALS PROVIDED & STORAGE INFORMATION

Component	Quantity	Storage information
Microplate	96 wells	4°C
Standard	1 vial (Lyophilized)	4°C
Assay Buffer	4 X 30 ml (Ready to use)	4°C
Reaction Buffer	16 ml (Ready to use)	4°C
Substrate	1 vial (Lyophilized)	4°C
Reaction Dye	1 vial (Lyophilized)	4°C
Reaction Dye Diluent	2 ml (Ready to use)	4°C

The kit is shipped at room temperature. Store the Reagent A at room temperature and all other reagents at 4°C. Shelf life of 12 months after receipt.

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 593 nm
- Pipettes and pipette tips
- Deionized or distilled water

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Briefly spin down the reagents before use.
- It is highly recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.
- All reagents should be warmed to room temperature before use.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g at 2-8°C. Remove serum and assay immediately or aliquot and store samples at ≤ -20°C or below. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using heparin as an anticoagulant (EDTA is a kind of iron chelators, and it interferes with this assay and should be avoided to use in sample preparation). Centrifuge for 15 minutes at 1000 x g at 2-8°C within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -

20°C or below. Avoid repeated freeze-thaw cycles.

Tissue or cell samples- Weigh out 0.1 g of tissue, then tissues are homogenized with 1 ml Assay buffer on ice. Centrifuge homogenized tissues at 12,000 g for 20 minutes 4°C. Collect the supernatant into a new centrifuge tube and keep it on ice for detection. Assay immediately or aliquot and store samples at ≤ -20°C or below. Avoid repeated freeze-thaw cycles

Cell Culture Supernatants, urine - Remove particulates by centrifugation and aliquot & store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Note:

1. *Samples should be clear and free of precipitates or turbidity. If not, centrifuge or filter to clarify samples prior to assay.*
2. *DO NOT use EDTA as the anticoagulant for plasma samples.*
3. *The sample also can not contain DTT, Mercaptoethanol, Tween, Triton and NP-40.*

REAGENT PREPARATION

- Standards: Reconstitute the standard with 1 ml distilled water to yield a stock concentration of 50 mmol/L. Allow the stock standard to sit for few minutes with gentle agitation to make sure the standard is dissolved completely before making serial dilutions. The distilled water serves as bland (0 mmol/L). Add 100 µl of stock standard (50 mmol/L) into 900 µl of distilled water to yield a working Standard concentration of 5 mmol/L. Store the reconstituted standard stock at 4°C.

- Substrate: Reconstitute the Substrate with 1.5 ml of distilled water. Allow the Substrate to sit in the tube for few minutes with gentle agitation to make sure the Substrate is dissolved completely before use. Store the reconstituted Substrate at 4°C.
- Reaction Dye: Reconstitute the Reaction Dye with 2 ml of Reaction Dye diluent. Allow the Reaction Dye to sit for few minutes with gentle agitation to make sure the Reaction Dye is dissolved completely before use. Store the reconstituted Reaction Dye at 4°C.

ASSAY PROCEDURE

Standards and samples should be assayed in at least duplicates.

Perform 2-fold serial dilutions of the top standards to make the standard curve.

1. Add 160 µl of Reaction Buffer into wells.
2. Add 5 µl per well of working standard, distilled water (blank) and samples in duplicates into appropriate wells of the plate.
3. Add 20 µl of Reaction Dye into per wells.
4. Gently tap plate to mix thoroughly.
5. Add 15 µl of Substrate into per wells.
6. Incubate the plate for 5 min at room temperature
7. Read O.D. with a microplate reader at 593 nm (510-630 nm) immediately.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards and samples.

2. Unit Definition: One unit of Total Antioxidant Capacity is defined as the sample generates 1 µmol of Fe2+ per minute.

3. Calculation:

A. Definition:

$C_{Protein}$: the protein concentration, mg/ml;

$C_{Standard}$: the protein concentration, 5 mmol/L = 5 µmol/ml

W: the weight of sample, g;

N: the quantity of cell or bacteria, $N \times 10^4$

$V_{Standard}$: the volume of the reaction, 0.005 ml;

V_{Sample}: the volume of sample, 0.005 ml;

V_{Assay}: the volume of Assay buffer, 1 ml.

T: the reaction time, 5 minutes.

B. Formula:

a). According to the protein concentration of sample

$$TAC\ (U/mg) = [(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank})] / [(OD_{Standard} - OD_{Blank}) \times (V_{Sample} \times C_{Protein}) \times T]$$

$$= (OD_{Sample} - OD_{Blank}) / [(OD_{Standard} - OD_{Blank}) \times C_{Protein}]$$

b). According to the weight of sample

$$TAC\ (U/g) = [(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank})] / [(OD_{Standard} - OD_{Blank}) \times (W \times V_{Sample} / V_{Assay}) \times T]$$

$$= (OD_{Sample} - OD_{Blank}) / [(OD_{Standard} - OD_{Blank}) \times W]$$

c). According to the quantity of cells or bacteria

$TAC\ (U/10^4\ cell) = [(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank})] / [(OD_{Standard} - OD_{Blank}) X (N \times V_{Sample} / V_{Assay}) X T]$

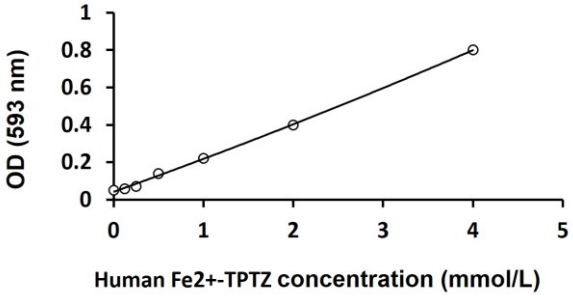
$= (OD_{Sample} - OD_{Blank}) / [(OD_{Standard} - OD_{Blank}) X N]$

d). According to the volume of serum, plasma

$TAC\ (U/ml) = [(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank})] / [(OD_{Standard} - OD_{Blank}) X V_{Sample} X T]$

$= (OD_{Sample} - OD_{Blank}) / [(OD_{Standard} - OD_{Blank})]$

EXAMPLE OF TYPICAL DATA



QUALITY ASSURANCE

Sensitivity

Detection Range: 0.05 mmol/L - 5 mmol/L