Total Antioxidant Capacity (TAC)

Colorimetric Assay kit

(96 Tests)

Zellbio GmbH (Germany)

CAT No. ZX-44109-96

www.zellx.de

Sample Types Validated for:

Serum, Plasma, Urine, Teas, Fruit Juices, Beer, Cider, Cell Lysates, Herbal and Fruit Extracts

!!! Caution: This product is for Research Use Only. Not for *in-vitro* Diagnostics !!!

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Please read this insert completely prior to using the product.

Krantorweg 48c, 13503 Berlin
Tel.: +49(0)30 81309085 | Fax: +49(0)30 81309086
E-Mail: support@zellx.de | Web: www.zellx.de

Introduction

Background

Antioxidants or free radical scavengers are the molecules which prevent free radicals and other potentially toxic oxidizing species from damaging cells and organisms. The source of antioxidants are either endogenous, i.e. produced by our body, or exogenous, i.e.. obtained via nutrition. Endogenous antioxidants are classified into two subgroups of enzymatic such as dismutase, reductase, catalase, peroxidase, etc. and non-enzymatic such as glutathione, bilirubin, metallothioneins, and uric acid. However, as the amount of free radicals is often higher than the capacity of endogenous antioxidants to detoxify, it is necessary to obtain some antioxidants from external sources (vitamin A, vitamin C, and vitamin E) to balance the disequilibrium.

As antioxidants are different in their reducing capacity, the Ferric Reducing Antioxidant Power (FRAP) is utilized to evaluate the reducing power of antioxidants in comparison to ascorbic acid as a standard. The antioxidant capacity of biological samples is considered as an indicator of the overall potential of body against undesired effects of reactive oxygen species (ROS) and oxidative stress-related diseases.

Assay principle

The ZellX® Total Antioxidant Capacity assay is designed to quantitatively evaluate the antioxidant status in a variety of samples. The Kit measures the capacity of both small molecule antioxidants and proteins to reduce the ferric ions (Fe^{3+}) of FRAP Color Solution to ferrous ions (Fe^{2+}) under acidic conditions. The reaction generates a blue-colored product which is read at 560 nm, proportional to the total antioxidant capacity. A Ferrous Chloride standard is provided to create a standard curve for the assay.

General information

Materials supplied in the Kit

Component	*Quantity*
Ferrous Chloride Standard (10 mM)	45 µL
Ascorbic Acid Control	1 vial
Assay Buffer concentrate (10 X)	12.5 mL
FRAP Reagent A	700 µL
FRAP Reagent B	700 µL
Clear Half Area 96 Well Plate	1 plate

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Storage instruction

All reagents should be stored at 4° C until the expiration date of the kit.

Materials required but not supplied

Deionized water (diH_2O)

Phosphate Buffer Saline (PBS)

Microplate/ELISA reader capable of reading optical absorption at 560 nm

Centrifuge, Vortex mixer

Precision pipettes, multichannel pipette and disposable pipette tips

Disposable 1.5-2 mL microtubes for sample preparation

Precautions

This kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product.

Solutions containing sodium azide will yield an instantaneous colored product with the FRAP Color Solution. Therefore no buffers or solutions containing azide can be measured using this kit.

General remarks

- Equilibrate all kit components to room temperature (RT) 30 minutes before use.
- The instruction must be strictly followed. The reading of Microplate/ELISA reader must be set as at the appropriate wavelength of determining the experiment result.
- Pipette tips should not be used more than once in order to avoid cross contamination.
- Reagents of different batches should not be mixed or used after their expiration dates.

Assay protocol

Reagent preparation

i. **Assay Buffer:** Prepare a 1:10 dilution of Assay Buffer Concentrate with diH_2O (1 part Assay Buffer Concentrate and 9 parts diH_2O), and mix well. Assay Buffer can be stored at 4°C for up to 3 months.

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ii. **Ascorbic Acid Working Solution:** Add 200 µL Assay Buffer to the Ascorbic Acid Control vial and vortex it well (The vial should be mixed for 5 minutes). Unused reconstituted control solution should be aliquoted at 50 µL per vial to minimize freeze-thaw cycles and stored at -20°C.

iii. **FRAP Color Solution:** Add 625 µL of FRAP Reagent A and 625 µL of FRAP Reagent B to 6.25 mL Assay Buffer and vortex well. **FRAP Color Solution should be used within 2 hours of preparation.**

Note: Any significant blue color in the prepared Color Solution may indicate contamination of the Assay Buffer, Reagent A or B.

Sample preparation

Samples must be diluted in Assay Buffer. Dilutions should be made to ensure that the activity level of samples fall within the standard curve range. As ascorbic acid is unstable at neutral pH, it is recommended that samples (fruit juices, wine, extracts of tea, herbs, candies, drinks, and dried food extracts) are diluted with Assay Buffer which has a lower pH.

Serum and plasma samples must be diluted ≥ 1:2 with Assay Buffer prior to conducting assay. The recommended samples for analysis of blood samples are serum, EDTA- or heparin-treated plasma.

Urine samples must be diluted ≥ 1:10 with Assay Buffer prior to measurement.

All the samples must be used within 2 hours of dilution

Standard preparation

- Prepare a 1:10 dilution of $FeCl_2$ Standard with Assay Buffer (mix 20 µL of standard with 180 µL of Assay Buffer), and label as the Standard No.6 (1000 µM).
- Apply series of other dilutions as described in the table.
- The Assay Buffer is used as the 0 µM standard.

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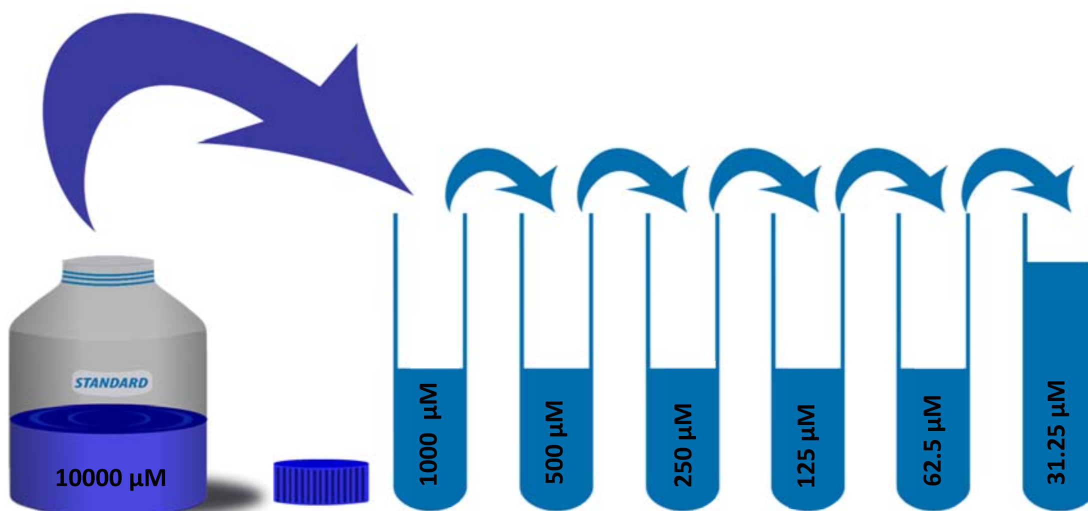
No.	Concentration	Material needed
Standard No.6	1000 µM	20 µL $FeCl_2$ Standard + 180 µL Assay Buffer
Standard No.5	500 µM	100 µL Standard No.6 + 100 µL Assay Buffer
Standard No.4	250 µM	100 µL Standard No.6 + 100 µL Assay Buffer
Standard No.3	125 µM	100 µL Standard No.6 + 100 µL Assay Buffer
Standard No.2	62.5 µM	100 µL Standard No.6 + 100 µL Assay Buffer
Standard No.1	31.25 µM	100 µL Standard No.6 + 100 µL Assay Buffer
Standard No.0	0 µM	100 µL Assay Buffer



All standard must be used within 2 hours of preparation

Assay Procedure

1. Pipet 20 µL of either samples or standards into duplicate wells in the plate.
2. Pipet 20 µL of Assay Buffer as the zero standard.
3. Add 20 µL of Ascorbic Acid Working Solution into duplicate wells as an optional control.
4. Add 75 µL of FRAP Color Solution to each well using a multichannel pipet.
5. Incubate at room temperature (RT) for 30 minutes.
6. Read the optical density at 560 nm.

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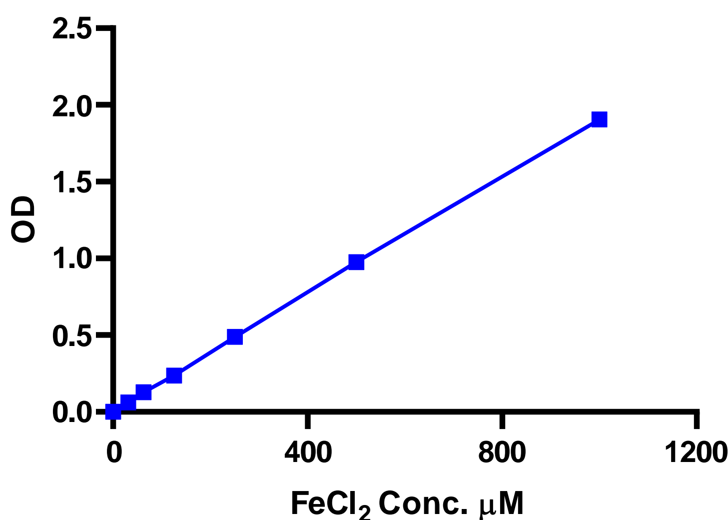
Calculation

- Average the duplicate OD readings for each standard and sample.
- Subtract the mean ODs for the zero standard from all OD values
  (for example if the OD value of zero standard, and standard 6 are 0.087, and 1.086 respectively; then the adjusted ODs equal 0 and 0.999 respectively.)
- Create a standard curve by reducing the data using the four parameter logistic curve (4PLC) fitting routine on the plate reader using the adjusted OD values
- The concentrations obtained should be multiplied by the dilution factor to obtain sample values.

Ascorbic Acid Control

The ascorbic acid control indicates that the FRAP Color Solution is producing acceptable color reaction in response to a typical antioxidant. Typical OD for this control should be about 50% of those produced by 1000 µM $FeCl_2$ standard.



A typical standard curve of ZellX® TAC Assay kit

Run your own standard curves for calculation of results

Assay range

The limit of detection for ZellX® TAC assay was determined as 5.91 µM.

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Sensitivity

The sensitivity of the ZellX® TAC assay was determined as 8.06 µM.

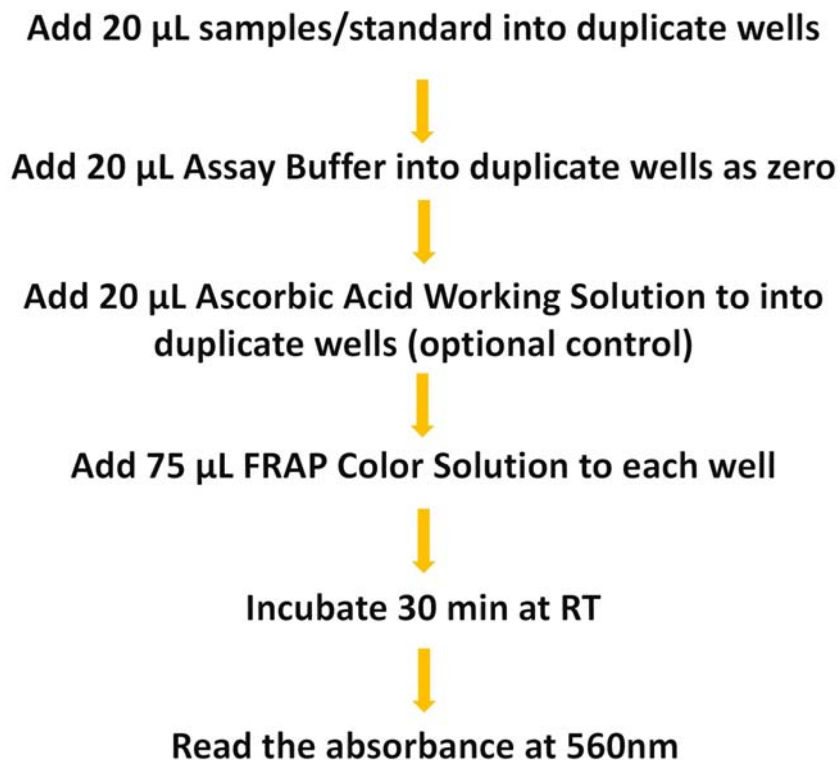
Precision

Intra-Assay Precision (Precision within an assay): 3 human serum samples were tested 20 times in an assay.

Inter-Assay Precision (Precision between assays): 3 human serum samples were tested in duplicate on 18 different assays over multiple days.

Item	*%CV*
Intra assay	3.0, 2.5, 2.2
Inter assay	2.9, 3.2, 4.2

Protocol summary



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