QuantiChrom™ FRAP Assay Kit (DFRAP-250)

Quantitative Colorimetric Antioxidant Determination

DESCRIPTION

FERRIC REDUCTION ANTIOXIDANT POTENTIAL (FRAP) is a measure of antioxidant capacity quantified by the antioxidant's potential to reduce ferric iron (III) to ferrous iron (II). Antioxidants protect cells from damage by reactive oxygen species that are produced by oxidation reactions. As oxidative stress contributes to the development of many diseases including Alzheimer's disease, Parkinson's disease, diabetes, rheumatoid arthritis and neurodegeneration, the use of antioxidants in pharmacology is intensively studied. Antioxidants are also widely used as dietary supplements and as preservatives in a wide range of products such as food, cosmetics, rubber and gasoline.

Simple, direct and high-throughput assays for antioxidant capacity find wide applications in research, food industry and drug discovery. BioAssay Systems' improved assay measures antioxidant potential in which Fe^{3+} is reduced by antioxidant to Fe^{2+}. The resulting Fe^{2+} specifically forms a colored complex with a chromogen. The color intensity at 590 nm is proportional to FRAP in the sample.

KEY FEATURES

Sensitive and accurate. Linear detection range: 0.5 - 180 µM Fe^{3+} reduction potential in 96-well plate assay.

Simple and high-throughput. The procedure involves addition of a single working reagent and incubation for 40 min. Can be readily automated as a high-throughput assay for thousands of samples per day.

Improved reagent stability and versatility. The optimized formulation has greatly enhanced reagent and signal stability.

APPLICATIONS

Direct Assays: FRAP in plant extracts, foods, vitamins, supplements, and biological samples such as serum, plasma, and urine.

KIT CONTENTS

Reagent A: 50 mL **Reagent C:** 4 mL

Reagent B: 4 mL **Standard:** 1 mL 1.8 mM Fe^{2+}

Storage conditions. The kit is shipped at room temperature. Store Reagent A at room temperature and other reagents at 4°C. Shelf life: 12 months after receipt.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

Sample Preparation: (1) Iron chelators (e.g. EDTA) interfere with this assay and should be avoided in sample preparation. (2) Samples containing iron will interfere with this assay; samples should be iron free in order to properly quantify FRAP. (3) Samples containing oxidants or reactive oxygen species (ROS) will interfere with this assay; samples should be free of oxidants and ROS in order to properly quantify FRAP. (4) Samples should be clear and free of precipitates or turbidity. If not, centrifuge or filter to clarify samples prior to assay. (5) Urine samples should be diluted 20-fold in dH_2O prior to assay (n = 20). (6) Serum and plasma samples should be diluted 10-fold in dH_2O prior to assay (n = 10).

Procedure using 96-well plate:

1. Standards. Prepare 200 µL 180 µM Premix by mixing 20 µL 1.8 mM Fe^{2+} Standard and 180 µL distilled water. Dilute standards as follows:

No	Premix + H_2O	Vol (µL)	Fe^{2+} (µM)
1	100 µL + 0 µL	100	180
2	60 µL + 40 µL	100	108
3	30 µL + 70 µL	100	54
4	0 µL + 100 µL	100	0

Transfer 50 µL diluted standards and 50 µL sample into a clear flat bottom 96-well plate.

2. Prepare enough Working Reagent (WR) by mixing 20 volumes of Reagent A, 1 volume Reagent B and 1 volume Reagent C. Fresh reconstitution is recommended. Equilibrate to room temperature before assay.
3. Add 200 µL WR to wells. Tap plate to mix.
4. Incubate 40 min at room temperature and read optical density at 510-630 nm (peak absorbance at 590 nm).

Procedure using cuvette:

1. Prepare standards as in 96-well assay. Set up centrifuge tubes labeled Standards and Samples. Transfer 250 µL standards and samples to tubes.
2. Add 1000 µL WR to all tubes. Mix by vortexing. Incubate 40 min at room temperature.
3. Transfer to cuvettes and read OD at 590 nm (510 nm-630 nm).

CALCULATION

Subtract OD of water blank (Standard #4) from all other standard OD values and plot the OD against standard reduced iron concentrations. Determine the slope using linear regression fitting. FRAP of the sample is calculated as

$$[\text{FRAP}] = \frac{OD_{SAMPLE} - OD_{BLANK}}{\text{Slope } (\mu M^{-1})} \times n \quad (\mu M\ Fe^{3+}\ \text{reduction potential})$$

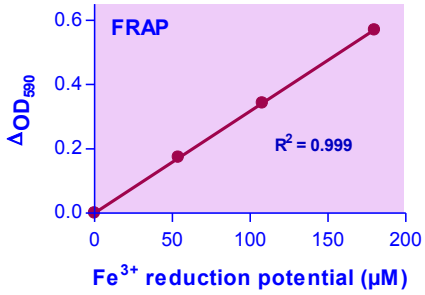
where OD_{SAMPLE} and OD_{BLANK} are OD values of the Sample and Water Blank (Standard #4) respectively. Slope is the slope of the standard curve and n is the dilution factor (e.g. n = 10 for serum samples).

Note: if the sample OD value is higher than OD for the 180 µM Fe^{2+} standard, dilute sample in water and repeat the assay. Multiply the results by the dilution factor.

Conversions: 1 µM Fe^{3+} reduction potential equals 0.5 µM Trolox equivalents.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices and accessories, clear bottom 96-well plates (e.g. Corning Costar) or cuvettes and plate reader or spectrophotometer capable of measuring OD at 510-630 nm.



Standard Curve in 96-well plate assay

LITERATURE

1. Dudonné, S. et al (2009). Comparative Study of Antioxidant Properties and Total Phenolic Content of 30 Plant Extracts of Industrial Interest Using DPPH, ABTS, FRAP, SOD, and ORAC Assays. Biochem. J. Agric. Food Chem. 57(5):1768-1774.
2. Müller, L. et al (2011). Comparative antioxidant activities of carotenoids measured by ferric reducing antioxidant power (FRAP), ABTS bleaching assay (αTEAC), DPPH assay and peroxyl radical scavenging assay. Food Chem. 129(1): 139-148.
3. Gohari, A.R. et al (2011). Antioxidant Activity of some Medicinal Species using FRAP Assay. J. Med. Plants. 10(37): 54-60.