Technical Bulletin

Ferric Reducing Antioxidant Power (FRAP) Assay Kit

Catalogue number MAK509

Product 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. The FRAP 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.

The linear detection range of the kit is 0.5 - 180 µM Fe^{3+} reduction potential. The kit is suitable for FRAP determination in plant extracts, foods, vitamins, supplements, and biological samples such as serum, plasma, and urine.

Components

The kit is sufficient for 250 colorimetric assays in 96-well plates

- Reagent A 50 mL
  Catalog Number MAK509A
- Reagent B 4 mL
  Catalog Number MAK509B
- Reagent C 4 mL
  Catalog Number MAK509C
- Standard (1.8 mM Fe^{2+}) 1 mL
  Catalog Number MAK509D

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (for example multichannel pipettor)
- Multiwell plate reader and cuvettes for procedure using cuvette.
- Clear, flat-bottom 96-well plates and 96-well plate absorbance reader for procedure using 96-well plate. Cell culture or tissue culture treated plates are not recommended.
- 1.5 mL microcentrifuge tubes

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped at room temperature. Store components at 2-8 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening. Equilibrate to room temperature prior use.

Note: (1) Iron chelators (for example 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.

Sample Preparation:

All Samples and Standards should be run in duplicate.

- Samples should be clear and free of precipitates or turbidity. If not, centrifuge or filter to clarify Samples prior to assay.
- Urine Samples should be diluted 20-fold in purified water prior to assay (DF = 20).
- Serum and plasma Samples should be diluted 10-fold in purified water prior to assay (DF = 10).

Colorimetric Standard Curve preparation:

1. Prepare a 180 µM Standard by mixing 20 µL of 1.8 mM Fe^{2+} Standard with 180 µL purified water.
2. Dilute the Standards as mentioned the table 1.

Table 1.
Preparation of Colorimetric Fe^{2+} Standards

Well No.	180 µM Standard	Purified Water	Fe^{2+} (µM)
1	100 µL	0 µL	180
2	60 µL	40 µL	108
3	30 µL	70 µL	54
4	0 µL	100 µL	0

Working Reagent Preparation

3. Freshly prepare enough reagent for the number of assays to be performed.
4. Mix 20 volumes of Reagent A, 1 volume of Reagent B and 1 volume of Reagent C.

Measurement

Using 96-well plate:

1. Transfer 50 µL of diluted Standard and Samples in duplicate to separate wells of a clear, flat-bottom well plate.
2. Add 200 µL working reagent and tap plate to mix thoroughly.
3. Incubate for 40 min at room temperature and read optical density (OD) at 510-630 nm (peak absorbance at 590 nm).

Using cuvette:

1. Prepare Standards as described in Table 1. Set up centrifuge tubes labeled Standards and Samples.
2. Transfer 250 µL Standard and Samples to appropriately labeled tubes.
3. Add 1000 µL working reagent and vortex to mix. Incubate for 40 min at room temperature. Transfer to cuvette and read OD at 590 nm (510 nm-630 nm).

Results

1. Calculate ΔOD by subtracting the reading (OD) of Standard #4 (Blank) from the remaining Standard reading values.
2. Plot the ΔOD against the standard concentrations and determine the slope of the standard curve.
3. Calculate the FRAP of sample using the below equation:

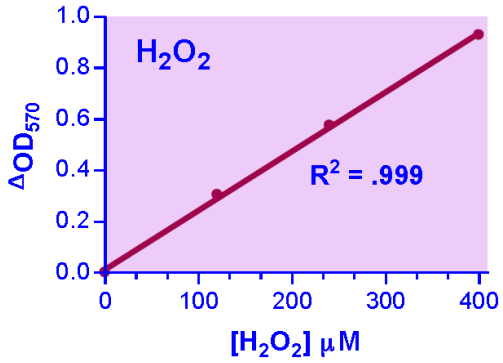
[FRAP] (µM Fe^{3+} reduction potential)=

$$\frac{R_{Sample}-R_{Blank}}{Slope} \times DF$$

where:
R_{Sample} = OD reading of Sample
R_{Blank} = OD reading of Blank
DF = Sample dilution factor
(DF = 1 for undiluted 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 (DF).

Figure 1.
Typical FRAP Standard Curve in purified water.



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