Ferric reducing antioxidant power (FRAP) Assay Kit

ARG83614 Ferric reducing antioxidant power (FRAP) Assay Kit is a detection kit for the quantification of Ferric reducing antioxidant power (FRAP).

Catalog number: ARG83614

Package: 100 assays

TABLE OF CONTENTS

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PRINCIPLE OF THE ASSAY

ARG83614 Ferric reducing antioxidant power (FRAP) Assay Kit is a quantitative assay for measuring the antioxidant potential within a sample. Ferric iron (Fe3+) is initially reduced by electron-donating antioxidants present within the sample to its ferrous form (Fe2+). The ironcolorimetric probe complex develops a dark blue color product upon reduction which can be measured at 540-600 nm (see Figure 1). Samples can be compared to the iron standard for determining antioxidant potential. This assay is analytically sensitive to approximately 4 µM of Fe2+ iron equivalents, or a FRAP value of 2.

MATERIALS PROVIDED & STORAGE INFORMATION

Upon receipt, store all Component at 4°C. Use the kit before expiration date.

Component	Quantity	Storage information
Iron (II) Standard (500 mg)	100 µl	4°C
Iron Chloride Solution	500 µl	4°C
Probe	50 µl	4°C
5X Assay Buffer	30 ml	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- 96-well microtiter plates
- Pipettes and pipette tips
- Deionized or distilled water
- Centrifuge spin filter

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid sample.
- Upon receipt, store all Component at 4°C. Use the kit before expiration date.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Change pipette tips between the addition of different reagent or sample.

SAMPLE COLLECTION & STORAGE INFORMATION

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

Cell Culture Supernatants- Remove particulates by centrifugation for 10 min at 1500 x g at 4°C and aliquot & store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Serum- Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes. Remove the yellow serum supernatant without disturbing the white buffy layer. Samples should be tested immediately or frozen at-80°C.

Plasma- Collect blood sample and add to a blood collection tube containing heparin as the anticoagulant. Centrifuge at 3,000 rpm for 10-15 minutes at 4ºC. Remove the upper yellow plasma supernatant layer without disturbing the white buffy coat (leukocytes). Samples should be tested immediately or frozen at-80°C.

Cell Lysate- Lyse 1-2 x 106 cells/mL by sonication or multiple freeze-thaw cycles in 4 volumes of cold 1X Assay Buffer. Centrifuge at 12,000 rpm for 15 minutes at 4ºC and remove insoluble cell material. A high concentration of protein may interfere with the assay. In this case, filter the sample with a 10kDa MWCO centrifugal filter before assaying (to reduce protein interference and turbidity). Test samples immediately or store at-80°C.

Tissue Lysate- Homogenize/sonicate approximately 10 mg of tissue in 1-2 mL cold 1X Assay Buffer. Centrifuge the homogenate at 12,000 rpm for 15 minutes at 4ºC and collect the supernatant. A high concentration of protein may interfere with the assay. In this case, filter the sample with a 10kDa MWCO centrifugal filter before assaying (to reduce protein interference and turbidity). Test samples immediately or store at-80°C.

Food extracts- Wash uncooked food (e.g., fruits or vegetables) in water and homogenize 5-10g (fresh wet weight) in 100 mL distilled water for 30 seconds. Filter homogenate and prepare dilutions in deionized water, 70% acetone, or 50% ethanol as necessary.

REAGENT PREPARATION

- **1x Assay Buffer** - Dilute the **5x Assay Buffer** into Deionized Water to yield **1x Assay Buffer**. The **1x Assay Buffer** is stable for up to 6 months at 2-8°C.
- **Working Detection Reagent**- Prepare this reagent immediately prior to use and use it within 20 min after preparation. Probe 1:10 and the Iron Chloride Solution 1:1000 in **1x Assay Buffer**.
- **Standards:** Weight and dissolved the Standard Prepare Iron (II) Standards for a 10 mg/ml (36 mM) solution in deionized water. Then, dilute this 36 mM stock to 1 mM in deionized water. before use by diluting in Deionized Water according to the Table below.

Standard tube	Iron (II) conc. (µM)	Deionized Water (µL)	Iron (II) Standard (1000 µM) (µL)
S1	500	500	500 (10 mMIron (II))
S2	250	500	500 of S1
S3	125	500	500 of S2
S4	32.5	500	500 of S3
S5	31.3	500	500of S4
S6	15.6	500	500 of S5
S7	73.8	500	500 of S6
S8	3.9	500	500
S0	0	500	500

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-25°C) before use. Standards and sample should be assayed in duplicates.

1. Add 100 µl of diluted sample or each diluted Standard into respective wells of the 96-well plate.
2. Add 100 µl of **Working Detection Reagent** to each well.
3. Read the absorbance with a plate reader at O.D. 540-600 nm.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and samples.
2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)

6. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Ferric reducing antioxidant power (FRAP). One should use the data below for demonstration only and cannot be used in place of data generations at the time of assay.