SIGMA-ALDRICH®

sigma-aldrich.com

3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Ferric Reducing Antioxidant Power (FRAP) Assay Kit

Catalog Number **MAK369**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

Ferric reducing antioxidant power (FRAP) assay is a widely used method that uses antioxidants as reductants in a redox-linked colorimetric reaction, wherein Fe^{3+} is reduced to Fe^{2+}. Ferric (Fe^{3+}) to ferrous (Fe^{2+}) ion reduction at low pH causes formation of a colored ferrous-probe complex from a colorless ferric-probe complex. Antioxidants are molecules which act as reducing agents by donating electrons to free radicals to stabilize them and minimize the damage caused by free radicals to DNA, cells, and organ systems. Antioxidants include substances such as polyphenols, flavonoids, vitamins, and enzymes like glutathione peroxidase and superoxide dismutase. They are known to have beneficial health effects such as lowering the risk of cancer, heart disease, and neurodegenerative disorders and are abundantly found in plants, fruits, vegetables, beverages, and natural products.

The Ferric Reducing Antioxidant Power (FRAP) Assay Kit provides a quick, sensitive, and easy way for measuring antioxidant capacity of various biological samples. The assay is high-throughput adaptable and can detect antioxidant capacities as low as 0.2 mM Fe^{2+} equivalents.

The kit is suitable for the measurement of antioxidant capacity in fruits, vegetables, beverages (like tea and wine), food products, plant extracts, herbal products, serum, and plasma.

Components

The kit is sufficient for 200 colorimetric assays in 96 well plates.

Component	Amount
FRAP Assay Buffer Catalog Number MAK369A	50 mL
FRAP Probe Catalog Number MAK369B	4 mL
$FeCl_3$ Solution Catalog Number MAK369C	4 mL
Ferrous Standard (2 mM) Catalog Number MAK369D	1 mL
FRAP Positive Control Catalog Number MAK369E	0.1 mL

Reagents and Equipment Required but Not Provided.

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Spectrophotometric multiwell plate reader, capable of 37 °C temperature setting
- Clear flat-bottom 96 well plates

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at 2–8 °C, protected from light. Briefly centrifuge small vials prior to opening.

Preparation Instructions

Reagent Preparation

FRAP Assay Buffer: Warm to room temperature prior to use.

Ferrous Standard (2 mM): Aliquot and store at 2–8 °C. Keep on ice while in use.

FRAP Positive Control: Aliquot and store at 2–8 °C, protected from light. Keep on ice while in use.

Procedure

Sample Preparation

A variety of fruit, vegetable, and plant samples, beverages as well as serum and plasma can be used with this assay. Fruit, vegetable and plant extractions can be done using acid-methanol (e.g., a mixture of methanol:ultrapure water:1 M HCl at a ratio of 70:29.5:0.5), acid-ethanol, or acetone extraction methods. Users can use the extraction methods of their choice for their particular samples with proper dilutions to ensure the values fall within the standard curve range. Do not use FRAP Assay Buffer for extraction of samples. Fruit and vegetable juices, herbal products, and freeze-dried fruits solubilized in suitable solvents, beverages such as wines, green tea, and coffee can also be used directly with appropriate dilutions while making sure potential interfering substances do not give a significant background. For additional details about extraction methods, see references 1-3.

For unknown samples, perform a pilot experiment and test several doses to ensure the readings are within the Standard Curve range. Also ensure that the reaction is complete within 60 minutes for the absorbance readings. For samples exhibiting significant background, prepare parallel sample well(s) as background controls.

Add 10 μL of sample per well. For the positive control, add 4 μL of the FRAP Positive Control plus 6 μL of FRAP Assay Buffer into desired well(s).

Standard Curve Preparation

Prepare Ferrous Standards in desired wells of a clear flat-bottom 96 well plate according to Table 1.

Table 1.
Preparation of Ferrous Standards

Well	Ferrous Standard (2 mM)	FRAP Assay Buffer	Ferrous (nmol/well)
1	0 μL	10 μL	0
2	2 μL	8 μL	4
3	4 μL	6 μL	8
4	6 μL	4 μL	12
5	8 μL	2 μL	16
6	10 μL	–	20

Reaction Mix

Mix enough reagents for the total number of wells to be assayed including standards, samples, positive control, and background control(s). For each well, prepare 190 μL of Reaction Mix according to Table 2.

Table 2.
Preparation of Reaction Mix

Reagent	Reaction Mix	Background Control Mix
FRAP Assay Buffer	152 μL	171 μL
$FeCl_3$ Solution	19 μL	19 μL
FRAP Probe	19 μL	–

Mix and add 190 μL of the Reaction Mix to each well containing the standards, positive control, and test samples. For background correction, add 190 μL of Background Control Mix (without FRAP probe) to sample background control well(s) and mix well.

Measurement

Measure absorbance immediately at 594 nm (A_{594}) in kinetic mode for 60 minutes at 37 °C. Use the absorbance values obtained at 60 minutes for further calculations (ensure the reaction is complete at 60 minutes). If desired, the Ferrous Standard Curve can be read in endpoint mode (i.e., at the end of the 60 minute incubation time).

Results

1. Subtract the 0 nmol Standard reading from all Standard Curve readings.
2. Plot the Ferrous Standard Curve.
3. If the sample background control reading is significant, subtract the background control reading from its paired sample reading.
4. Compare the sample A_{594} values to the Ferrous Standard Curve to get nmol of reduced Ferrous ions generated during the reaction (B).
5. Use the following calculation to determine mM Ferrous equivalents of the samples.

Sample FRAP or mM Ferrous Equivalent (nmol/μL or mM Fe^{2+} equivalents) =

$$\frac{B \times D}{V}$$

where:

B = Ferrous ammonium sulphate amount from Standard Curve (nmol)
D = Sample dilution factor
V = Sample volume added into the reaction well (μL)

Figure 1.
Typical Ferrous Standard Curve

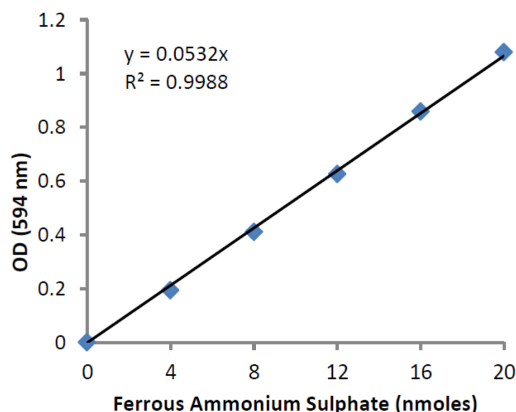
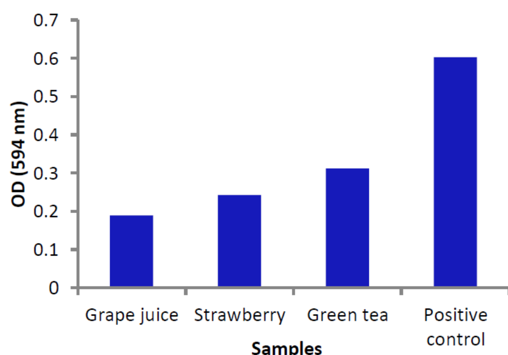
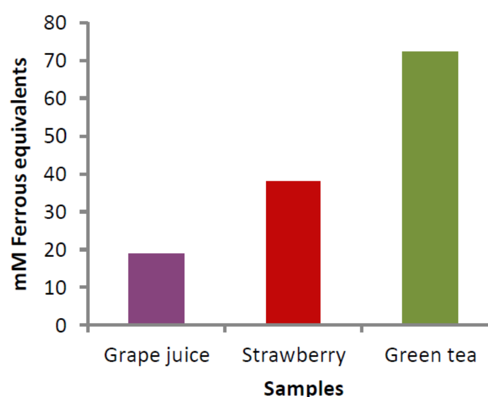


Figure 2.
Absorbance readings (A_{594}) of Samples



Absorbance readings (A_{594}) for positive control and 10 μL of diluted solutions of grape juice (50-fold dilution with ultrapure water), strawberry methanolic extract (extract made from 50 mg of freeze-dried strawberries in 5 mL of methanol and final solution diluted 80-fold with ultrapure water) and green tea (brewed for 5 minutes and diluted 120-fold with ultrapure water).

Figure 3.
FRAP (mM Ferrous equivalents) of Samples



FRAP (mM Ferrous equivalents) of grape juice, strawberry, and green tea. Assays were performed following the kit procedure.

References

1. Hidalgo, G.-I., and Almajano, M.P., Red Fruits: Extraction of Antioxidants, Phenolic Content, and Radical Scavenging Determination: A Review. Antioxidants (Basel), 19, 6(1) (2017).
2. Pisoschi, A.M. et al., Antioxidant Capacity Determination in Plants and Plant-Derived Products: A Review. Oxid. Med.Cell Longev., 2016, Volume 2016, Article ID 9130976, 36 pages
3. Stalikas, C.D., Extraction, separation, and detection methods for phenolic acids and flavonoids. J. Sep. Sci., 30(18), 3268-95 (2007).

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