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ab234626 Ferric Reducing Antioxidant Power (FRAP) Assay Kit (Colorimetric)

For the measurement of antioxidant capacity of various biological samples.

This product is for research use only and is not intended for diagnostic use.

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Table of Contents

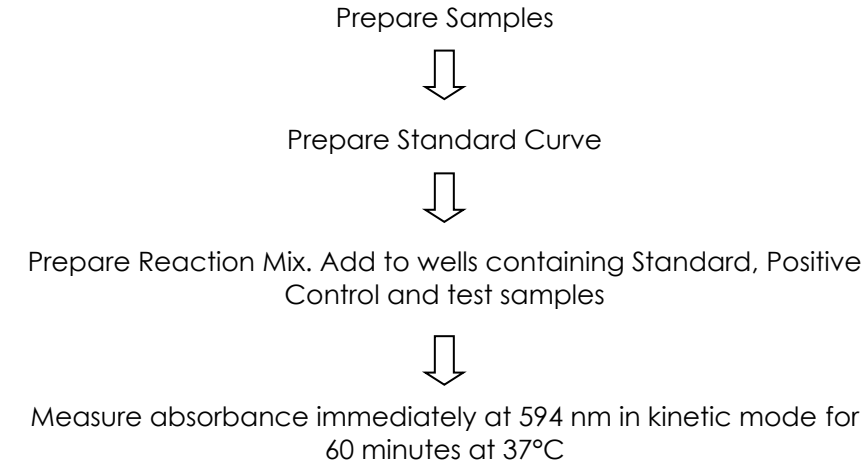
1. Overview 1
2. Materials Supplied and Storage 2
3. Materials Required, Not Supplied 2
4. General guidelines, precautions, and troubleshooting 3
5. Reagent Preparation 4
6. Standard Preparation 5
7. Sample Preparation 6
8. Assay Procedure 7
9. Data Analysis 8
10. Typical Data 9
11. Notes 11

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1. Overview

Ferric Reducing Antioxidant Power (FRAP) Assay Kit (Colorimetric) (ab234626) 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.

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.



2. Materials Supplied and Storage

Store kit at 4°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 2 months from receipt, if components have not been reconstituted.

Aliquot components in working volumes before storing at the recommended temperature.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
FRAP Assay Buffer	50 ml	4°C	4°C
FRAP Probe	4 ml	4°C	4°C
Ferric Iron Solution	4 ml	4°C	4°C
Ferrous Standard	1 ml	4°C	4°C
FRAP Positive Control	100 µl	4°C	4°C

PLEASE NOTE: Ferric Iron Solution was previously labelled as FeCl3 Solution. The composition has not changed.

3. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at O.D. 594 nm
- 96 well plate with clear flat bottom

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

5.1 FRAP Assay Buffer

Ready to use as supplied. Warm to room temperature before use.

5.2 FRAP Probe

Ready to use as supplied. Use within two months.

5.3 Ferric Iron Solution

Ready to use as supplied. Use within two months.

5.4 Ferrous Standard

Ready to use as supplied. Aliquot and store at 4°C. Use within two months. Keep on ice while in use.

5.5 FRAP Positive Control

Ready to use as supplied. Aliquot and store at 4°C. Use within two months. Keep on ice while in use. Protect from light.

6. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

1. Using the Ferrous Standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	Ferrous Standard (µL)	Assay Buffer (µL)	Final volume standard in well (µL)	End amount of Ferrous Standard in well (nmol/well)
1	0	20	10	0
2	4	16	10	4
3	8	12	10	8
4	12	8	10	12
5	16	4	10	16
6	20	0	10	20

Each dilution has enough standard to set up duplicate readings (2 x 10 µL).

7. Sample Preparation

General sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- Ensure reaction is complete at 60 minutes for the absorbance reading.
- We recommend that you use fresh samples for the most reproducible assay.

7.1 Samples:

1. A variety of fruit, vegetable and plant samples, beverages as well as serum and plasma can be used with this assay.
2. Fruit, vegetable and plant extractions can be done using acid-methanol (For e.g., Methanol: H_2O:1N HCl - 70:29.5:0.5), acid-ethanol or acetone extraction methods. Users can use the extraction method of their choice for their particular samples with proper dilutions to ensure the values fall within the standard curve range.
3. Fruit/vegetable juices, herbal products and freeze-dried fruits solubilized in suitable solvents, beverages such as wines, green tea, coffee can also be used directly with appropriate dilutions while making sure potential interfering substances do not give a significant background.
4. Add 10 µL of sample per well.
5. For the positive control, add 4 µL of the Positive Control per well into the desired well(s) and make up the volume to 10 µL with FRAP Assay Buffer.

Δ Note: *Do not use Assay Buffer for extraction of samples. Only to be used in the assay as directed.*

8. Assay Procedure

- Equilibrate the FRAP Assay Buffer to room temperature just prior to use and gently agitate. Keep other reagents on ice while in use.
- Assay all standards, controls and samples in duplicate.

Δ Note: *If you suspect your samples contain substance that can generate background, set up Sample Background Controls to correct for background noise.*

8.1 Reaction mix:

1. Prepare 190 µL of Reaction Mix and Background Mix for each reaction. Prepare a master mix to ensure consistency.

Component	Reaction Mix (µL)	Background Reaction Mix (µL)
FRAP Assay Buffer	152	171
FRAP Probe	19	19
Ferric Iron Solution	19	---

2. Add 190 µL of Reaction Mix into each standard, positive control and sample wells.
3. Add 190 µL of Background Reaction Mix into the background control sample wells.
4. Measure absorbance immediately at 594 nm in kinetic mode for 60 minutes at 37°C. Use the absorbance values obtained at 60 minutes for further calculations.

   Δ Note: *We recommend measuring the absorbance in kinetic mode, and choosing the values at 60 minutes after ensuring the reaction has reached completion. The Ferrous Standard Curve can be read in endpoint mode i.e. at the end of the incubation time (60 minutes).*

9. Data Analysis

Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.

1. Average the duplicate reading for each standard, control and sample.
2. Subtract the mean value of the blank (Standard #1) from all standards, controls and sample readings. This is the corrected absorbance.
3. If significant, subtract the sample background control from sample readings.
4. Plot the corrected values for each standard as a function of the final concentration of reduced Ferrous ions.
5. Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).
6. Apply the corrected sample O.D. reading to the standard curve to get Ferrous ammonium sulfate (B) amount in the sample wells.
7. Use the following calculation to determine mM Ferrous Equivalents of the samples:

$$\text{Sample FRAP or mM Ferrous Equivalents} = B \times \left(\frac{D}{V}\right)$$

Where:

B = Ferrous ammonium sulfate amount from Standard Curve (nmol).

V = Sample volume added in the sample wells (µL).

D = Sample dilution factor if sample is diluted to fit within the standard curve range (prior to reaction well set up).

10. Typical Data

Data provided **for demonstration purposes only**.

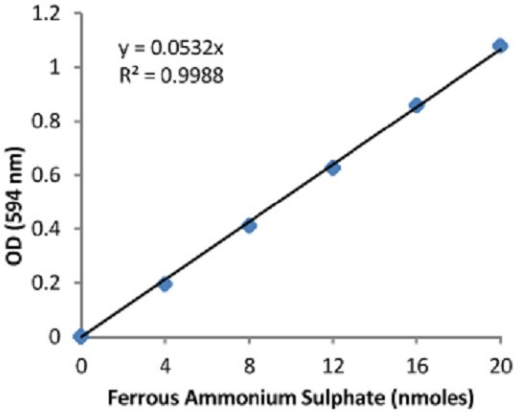


Figure 1. Ferrous standard curve.

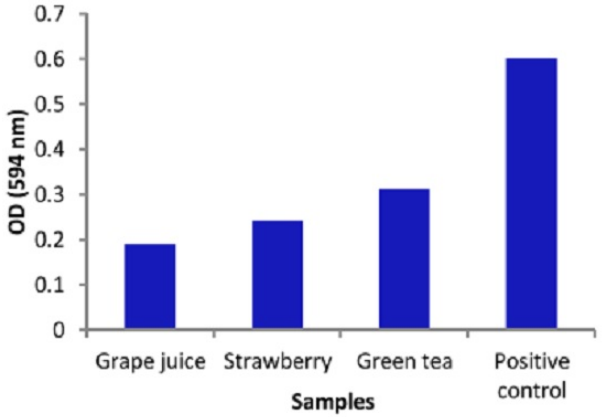


Figure 2. Absorbance readings for positive control and 10 µl diluted solutions of grape juice (1:50 dilution with dH_2O), strawberry methanolic extract (extract made from 50 mg of freeze-dried strawberries in 5 ml of extraction solvent and final solution diluted 1:80 times with dH_2O) and green tea (brewed for 5 minutes and diluted 1:120 times with dH_2O).

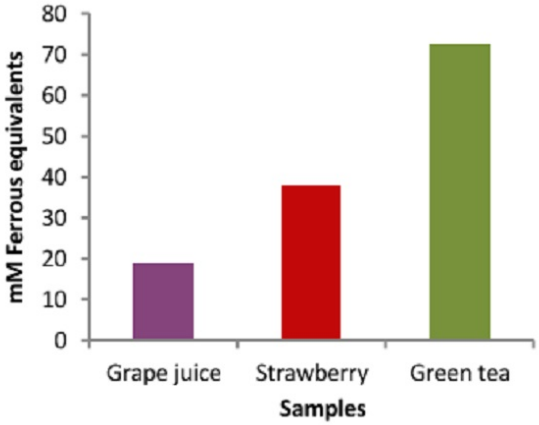


Figure 3. Absorbance readings for mM Ferrous equivalents or FRAP of grape juice, strawberry and green tea.

11. Notes

Technical Support

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