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

rev 02/20

(Catalog # K515-200; 200 assays; Store at 4°C)

I. Introduction:

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 the 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. **BioVision's 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.

Fe^{3+} - Probe + Reducing Antioxidant ⟶ Fe^{2+}- **Probe** **(λ = 594 nm)**

II. Application:

- Measurement of antioxidant capacity in fruits, beverages, food products, plants.

III. Sample Types:

- Fruits; vegetables; beverages like tea, wine; food products
- Plant extracts, herbal products
- Serum and plasma

IV. Kit Contents:

Components	K515-200	Cap Code	Part Number
FRAP Assay Buffer	50 ml	WM	K515-200-1
FRAP Probe	4 ml	Blue/Amber	K515-200-2
$FeCl_3$ Solution	4 ml	Red/Amber	K515-200-3
Ferrous Standard (2 mM)	1 ml	Yellow	K515-200-4
FRAP Positive Control	0.1 ml	Violet	K515-200-5

V. User Supplied Reagents and Equipment:

- Clear 96-well plate with flat bottom
- Multi-well spectrophotometer (plate reader)

VI. Storage Conditions and Reagent Preparation:

Store kit at 4°C, protected from light. Briefly centrifuge the small vials prior to opening. Read the entire protocol before performing the assay.

- **FRAP Assay Buffer:** Warm to room temperature (RT) before use. Store at 4°C.
- **FRAP Probe:** Store at 4°C. Use within two months.
- **$FeCl_3$ Solution:** Store at 4°C. Use within two months.
- **Ferrous Standard (2 mM):** Aliquot and store at 4°C. Keep on ice while in use. Use within two months.
- **FRAP Positive Control:** Aliquot and store at 4°C. Protect from light. Use within two months. Keep on ice while in use.

VII. Ferric Reducing Antioxidant power Colorimetric Assay Protocol:

1. **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 (For e.g., Methanol:H_2O:1N HCl-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. 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. [For more details about the extraction methods, see references 1-3]. Add 10 µl of sample per well. For 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.

   Notes:

   a. Do not use FRAP Assay Buffer for extraction of samples. Only to be used in the assay as directed.
   b. For Unknown Samples, we suggest testing several dilutions to ensure the readings are within the Standard Curve range and the reaction is complete at 60 min for the absorbance reading.
   c. For samples exhibiting significant background, prepare parallel sample well(s) as Background Controls.

2. **Ferrous Standard Curve:** Add 0, 2, 4, 6, 8, 10 µl of 2 mM Ferrous Standard into a series of wells in 96 well clear plate to generate 0, 4, 8, 12, 16 and 20 nmol/well of Ferrous Standard. Adjust the volume to 10 µl/well with FRAP Assay Buffer.
3. **Reaction Mix:** Mix enough reagents for the number of assays to be performed. For each well, prepare 190 µl Reaction Mix containing:

	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 Standard, 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.

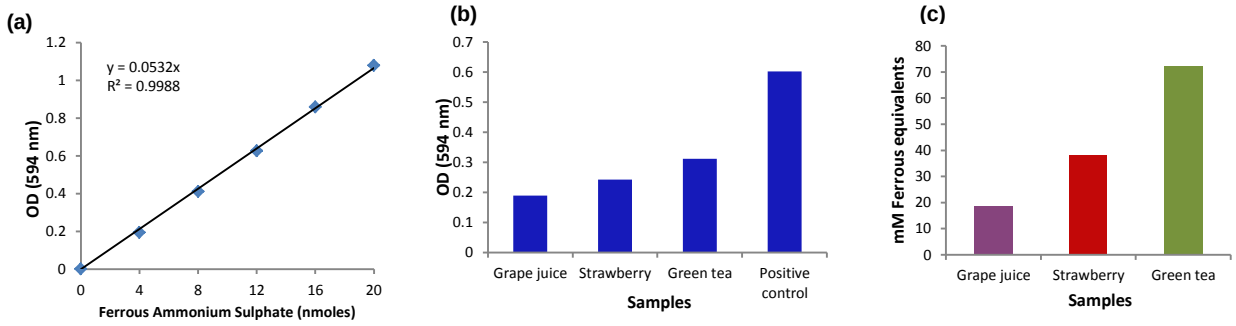
4. **Measurement:** Measure absorbance immediately at 594 nm in kinetic mode for 60 min at 37°C. Use the absorbance values obtained at 60 min for further calculations.

   Note: We recommend measuring the absorbance in kinetic mode, and choosing the values at 60 min 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 min)].

5. **Calculation:** Subtract the 0 nmol Standard reading from all Standard Curve readings. Plot the Ferrous Standard Curve. If Sample Background Control reading is significant, subtract the Background Control reading from its paired sample reading. Apply the sample OD values to the Ferrous Standard Curve to get B nmol of reduced Ferrous ions generated during the reaction. Use the following calculation to determine mM Ferrous equivalents of the samples.

Sample FRAP or mM Ferrous Equivalents = B X D /V = nmol/µl **=** mM Fe^{2+} equivalents

Where: **B** = Ferrous ammonium sulphate amount from the Standard Curve (nmol).
D = Dilution Factor
V = Sample volume added into the reaction well (µl).



Figures: (a) Ferrous Standard Curve (b) 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 min and diluted 1:120 times with dH_2O) (c) mM Ferrous equivalents or FRAP of grape juice, strawberry and green tea. Assays were performed following the kit protocol.

VIII. Related Products:

Total Antioxidant Capacity (TAC) Colorimetric Assay Kit (K274)
Glutathione Fluorometric Assay Kit (K251)
Catalase Activity Colorimetric/Fluorometric Assay Kit (K773)
Glutathione (GSH/GSSG/Total) Fluorometric Assay Kit (K264)
Glutathione Colorimetric Assay Kit (K261)
Superoxide Dismutase (SOD) Activity Colorimetric Assay Kit (K335)

FOR RESEARCH USE ONLY! Not to be used on humans.

References:

1. Gádor-Indra Hidalgo and María Pilar Almajano. Red Fruits: Extraction of Antioxidants, Phenolic Content, and Radical Scavenging Determination: A Review. Antioxidants (Basel).2017, 19;6(1)
2. Pisoschi AM, Pop A, Cimpeanu C, Predoi G. 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 CD. Extraction, separation, and detection methods for phenolic acids and flavonoids. J Sep Sci. 2007, 30(18):3268-95.