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ARBOR ASSAYS™
Interactive Assay Solutions™

DetectX®

FRAP™
(Ferric Reducing Antioxidant Power) Colorimetric Detection Kit

2 Plate Kit Catalog Number K043-H1

Species Independent

Sample Types Validated:

Serum, Plasma, Urine, Teas, Fruit Juices, Beer, Cider, Cell Lysates, Herbal and Fruit Extracts

Covered under US, European and International Patents

Please read this insert completely prior to using the product.
For research use only. Not for use in diagnostic procedures.
Not for human diagnostic use.

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BACKGROUND

Reactive oxygen species (ROS)[1,2] are produced as a consequence of normal aerobic metabolism[3]. These "free radicals" (FR) are usually removed or converted into other products *in vivo* by an array of antioxidants. Antioxidants are typically chemically stable atoms and molecules, which have one (or rarely more) free electron/electrons in their electron envelope. Almost all biomolecules, but mainly biomembranes, proteins and nucleic acids, may be attacked by reactive free radicals. Free radicals are responsible for many pathological processes, or they can be generated as the result of the pathological stage and cause important secondary damage to biological systems and cells[1-5]. Connections between free radicals and some serious diseases, including Parkinson's and Alzheimer's diseases, atherosclerosis, myocardial infarction, and chronic fatigue syndrome, have been demonstrated. However, short-term oxidative stress, the unbalance between the formation and scavenging of the reactive oxygen species, may be important in the prevention of aging due to triggering the process known as mitohormesis. On the average, 65–70 % of the population is excessively impacted by oxidative stress caused by FRs. In 1996 Iris Benzie and Sean Strain published a simple assay to measure antioxidant power[6]. The original demonstration of the power of this assay to measure antioxidant potential in serum and plasma has been extended to the antioxidant power of certain foods[7], teas[8], and fungi.

The protective system of organisms is based on the activity of specific enzymes (especially superoxide dismutase, glutathione peroxidase, catalase, glutathione reductase) as well as non-enzymatic compounds with antioxidant activity (ß-tocopherol, L-ascorbic acid, glutathione, coenzyme Q10, flavonoids, albumin and other molecules). Excess production of reactive oxygen species can also lead to inflammation, premature aging disorders, and several disease states, including cancer, diabetes, and atherosclerosis. Organisms have developed complex antioxidant systems to protect themselves from oxidative stress, however, excess ROS can overwhelm the systems and cause severe damage.

1. Halliwell, B. (1994). Free radicals and antioxidants: A personal view. *Nutrition Reviews, 52*(8), 253-265.
2. Gutteridge, J. M. C. (1994). Biological origin of free radicals, and mechanisms of antioxidant protection. *Chemico-Biological Interactions, 91*(2-3), 133–140.
3. Gutteridge, J. M. C. (1995). Lipid peroxidation and antioxidants as biomarkers of tissue damage. *Clinical Chemistry, 41*(12), 125–126.
4. Frei, B., et al. (1988). Antioxidant defenses and lipid peroxidation in human blood plasma. *Proceedings of the National Academy of Sciences, USA, 85*, 9748–9752.
5. Frei, B., et al. (1989). Ascorbate is an outstanding antioxidant in human blood plasma. *Proceedings of the National Academy of Sciences, USA, 86*, 6377–6381.
6. Benzie, I. F. & Strain, J.J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of ''antioxidant power'': The FRAP assay. *Analytical Biochemistry, 239*(1), 70-76.
7. Szeto, Y.T., et al. (2002). Total antioxidant and ascorbic acid content of fresh fruits and vegetables: Implications for dietary planning and food preservation. *British Journal of Nutrition, 87*, 55-59.
8. Benzie, I. F. & Szeto, Y. T. (1999). Total antioxidant capacity of teas by the ferric reducing/antioxidant power assay. *Journal of Agricultural and Food Chemistry*, *47*(2), 633-636.

ASSAY PRINCIPLE

The DetectX® Ferric Reducing Antioxidant Power (FRAP™) Detection kit is designed to quantitatively measure antioxidant status in a variety of samples. The assay measures the antioxidant ability from all species. Please read the complete kit insert before performing this assay. A Ferrous Chloride standard is provided to generate a standard curve for the assay and all samples should be read off of the standard curve. Samples are diluted in Assay Buffer and added to the wells. The FRAP Color Solution is made by mixing Reagent A and B with Assay Buffer. The FRAP Color Solution is added to all wells and the plate incubated at room temperature. Antioxidant power in the samples reacts with the FRAP Color Solution to generate a blue colored product which is read at 560 nm.

RELATED PRODUCTS

Kits	Catalog No.
Catalase Fluorescent Activity Kit	K033-F1
Catalase Colorimetric Activity Kit	K033-H1
Glutathione Fluorescent Detection Kits	K006-F1/F5
Glutathione Colorimetric Detection Kit	K006-H1
Glutathione Reductase Fluorescent Activity Kit	K009-F1
Hemoglobin Dual Range Detection Kit	K013-H1
Hydrogen Peroxide (H2O2) Fluorescent Detection Kit	K034-F1
Hydrogen Peroxide (H2O2) Colorimetric Detection Kit	K034-H1
Superoxide Dismutase (SOD) Activity Kit	K028-H1

SUPPLIED COMPONENTS

Clear 96 well Half Area Plates
Corning Costar Plate 3695.

2 Plates — Catalog Number X018-2EA

Ferrous Chloride Standard
Ferrous chloride at 10 mM in stabilizing solution.

90 µL — Catalog Number C152-90UL

Assay Buffer Concentrate
A 10X acetate buffer concentrate with stabilizers and preservatives that must be diluted with deionized or distilled water.

25 mL — Catalog Number X121-25ML

Ascorbic Acid Control
A vial containing 100 nmol Ascorbic Acid.

1 Vial — Catalog Number C150-1EA

FRAP Reagent A
FRAP Reagent A solution.

1.4 mL — Catalog Number C148-1.4ML

FRAP Reagent B
FRAP Reagent B solution.

1.4 mL — Catalog Number C149-1.4ML

STORAGE INSTRUCTIONS

All components of this kit should be stored at 4°C until the expiration date of the kit.

OTHER MATERIALS REQUIRED

Deionized water.

Repeater pipet with disposable tips capable of dispensing 75 µL.

96 well plate reader capable of reading optical absorption at 560 nm. Set plate parameters for a 96-well Corning Costar 3695 plate. See: www.arborassays.com/resources/#general-info for plate dimension data.

Software for converting optical density (OD) readings from the plate reader and carrying out four parameter logistic curve (4PLC) fitting. Contact your plate reader manufacturer for details.

PRECAUTIONS

As with all such products, this kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product.

Solutions containing sodium azide will yield an instantaneous colored product with the FRAP Color Solution. No buffers or solutions containing azide can be measured using this kit.

SAMPLE TYPES AND PREPARATION

Samples must be diluted in diluted Assay Buffer. Dilutions should be made to ensure that activity levels for samples fall within the standard curve range.

Serum and plasma samples must be diluted ≥ 1:2 in diluted Assay Buffer to be measured in this kit. The recommended samples for analysis of blood samples are serum, EDTA or heparin plasma.

Urine samples must be diluted ≥ 1:10 with diluted Assay Buffer prior to testing.

A variety of other samples can be run in the kit after suitable dilution including liquids such as fruit juices, wine, extracts of tea, herbs, candies, drinks, and dried food extracts. As ascorbic acid is unstable at neutral pHs it is recommended that extracts are diluted into the kit Assay Buffer which has a low pH. Please see references 6-8 on page 3.

In our evaluations, human serum and plasma samples had FRAP concentrations that ranged from approximately 700 to over 1,000 µM, with a mean level 858.7 µM. Human urine had levels from 2,547 to 7,410 µM with a mean of 5,331 µM. Pomegranate juice had a level of almost 82,300 µM.

Use all samples within 2 hours of perparation.

REAGENT PREPARATION

Assay Buffer

Dilute Assay Buffer Concentrate 1:10 by adding one part of the concentrate to nine parts of deionized water. Once diluted this is stable at 4°C for 3 months.

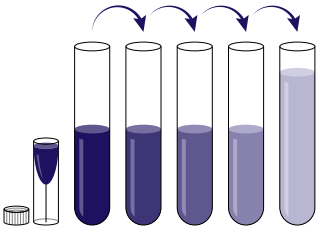
Ascorbic Acid Control

400 µL of diluted Assay Buffer should be added to the vial and vortexed. The vial should be mixed for 5 minutes. Unused reconstituted control solution should be aliquoted at 50 µL per vial to minimize freeze thaw cycles and stored at -20°C.

REAGENT PREPARATION (CONTINUED)

Standard Preparation

Standards are prepared by labeling test tubes #1 through #6. Add 180 µL of diluted Assay Buffer to tube #1 and pipet 100 µL of diluted Assay Buffer into the remaining tubes. Carefully add 20 µL of the Ferrous Chloride Standard Stock to tube #1 and vortex. Add 100 µL of the solution from tube #1 to tube #2 and vortex completely. Repeat the serial dilutions for tubes #3 through #6. The concentration of Ferrous Chloride in tubes 1 through 6 will be 1,000, 500, 250, 125, 62.5, and 31.25 µM $FeCl_2$.



	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Assay Buffer (µL)	**180**	100	100	100	100	100
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5
Volume of Addition (µL)	**20**	100	100	100	100	100
$FeCl_2$ Conc (µM)	1,000	500	250	125	62.5	31.25

Use all Standards within 2 hours of preparation.

FRAP Color Solution Preparation

Measure out diluted Assay Buffer into a clean container. Add FRAP Reagent A followed by FRAP Reagent B to the container and mix well to make FRAP Color Solution.

	1/2 Plate	1 Plate	1.5 Plates	2 Plates
Assay Buffer	3.4 mL	6.25 mL	10 mL	12.5 mL
FRAP Reagent A	340 µL	625 µL	1 mL	1.25 mL
FRAP Reagent B	340 µL	625 µL	1 mL	1.25 mL
FRAP Color Solution	4.08 mL	7.5 mL	12 mL	15 mL

Prepared FRAP Color Solution should be used within 2 hours. Note: Any significant blue color in the prepared Color Solution may indicate contamination of the Assay Buffer, Reagent A or B.

ASSAY PROTOCOL

We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine antioxidant status.

Use the plate layout sheet on the back page to aid in proper sample and standard identification.

1. Pipet 20 µL of samples or standards into duplicate wells in the plate.
2. Pipet 20 µL of diluted Assay Buffer into duplicate wells as the Zero standard.
3. Pipet 20 µL of diluted Ascorbic Acid Control into duplicate wells as an optional control.
4. Add 75 µL of the prepared FRAP Color Solution to each well using a repeater pipet.
5. Incubate at room temperature for 30 minutes.
6. Read the optical density at 560 nm.

CALCULATION OF RESULTS

Average the duplicate OD readings for each standard and sample. Create a standard curve by reducing the data using computer software capable of generating a four-parameter logistic curve (4PLC) fit. The sample concentrations obtained should be multiplied by the dilution factor to obtain neat sample values.

Or use the online tool from MyAssays to calculate the data:
www.myassays.com/arbor-assays-frap-(ferric-reducing-antioxidant-power)-detection-kit.assay

TYPICAL DATA

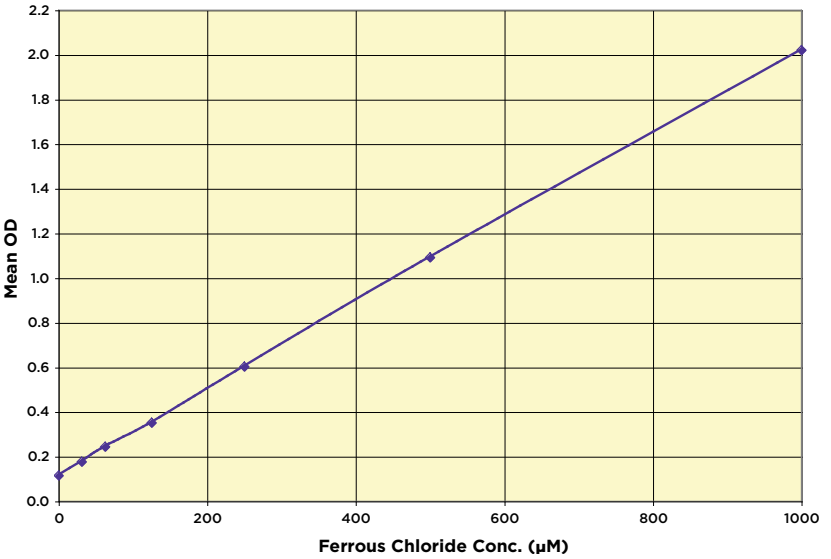
Sample	Mean OD	Net OD	$FeCl_2$ Concentration (µM)
Standard 1	2.021	1.905	1,000
Standard 2	1.092	0.976	500
Standard 3	0.604	0.488	250
Standard 4	0.352	0.236	125
Standard 5	0.244	0.128	62.5
Standard 6	0.177	0.061	31.25
Zero	0.116	0.0	0
Sample 1	1.976	1.860	974.3
Sample 2	0.553	0.437	225.0

Always run your own standard curves for calculation of results. Do not use this data.

Ascorbic Acid Control

This assay measures the ability of antioxidants to convert ferric to ferrous ions. The ascorbic acid control indicates that the FRAP Color Solution is producing acceptable color reaction in response to a typical antioxidant. Typical optical densities should be about 50% of those produced by 1,000 µM $FeCl_2$ standard.

TYPICAL STANDARD CURVE



Always run your own standard curves for calculation of results. Do not use this data.

VALIDATION DATA

Sensitivity

Sensitivity was determined by comparing the ODs for twenty wells run for each of the zero and lowest standard. The detection limit was determined at two (2) standard deviations from the zero along the standard curve.

Sensitivity was determined as 8.06 µM.

Limit of Detection

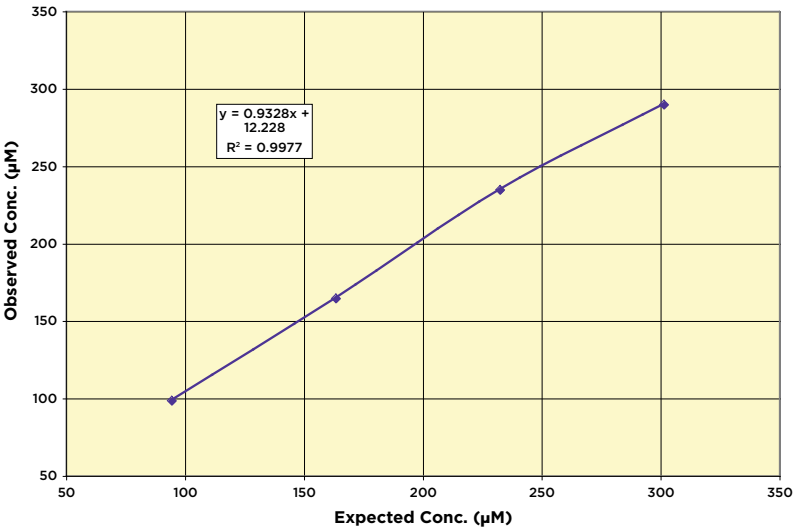
Limit of Detection was determined in a similar manner by comparing the OD's for twenty runs for each of the zero standard and a low concentration urine sample.

Limit of Detection was determined as 5.91 µM.

Linearity

Linearity was determined by taking two human samples, a diluted serum sample with a known high FRAP level and a diluted urine sample with a known lower FRAP level, and mixing them in the ratios given below. The measured $FeCl_2$ concentrations were compared to the expected values based on the ratios used.

Low Sample	High Sample	Expected Conc. (µM)	Observed Conc. (µM)	% Recovery
80%	20%	94.45	98.59	104.4
60%	40%	163.4	164.5	100.6
40%	60%	232.5	234.7	100.9
20%	80%	301.5	289.8	96.1
			Mean Recovery	**100.5%**



Intra Assay Precision

Three human samples diluted in Assay Buffer were run in replicates of 20 in an assay. The mean and precision of the calculated concentrations were:

Sample	$FeCl_2$ Conc. (µM)	%CV
1	953.0	2.2
2	597.7	2.5
3	236.2	3.0

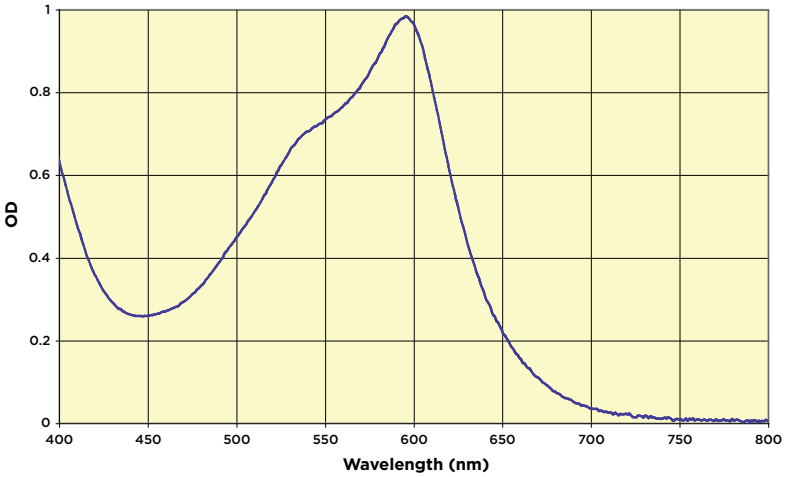
Inter Assay Precision

Three human samples diluted in Assay Buffer were run in duplicates in eighteen assays run over multiple days by three operators. The mean and precision of the calculated concentrations were:

Sample	$FeCl_2$ Conc. (µM)	%CV
1	951.2	2.9
2	561.2	3.2
3	232.2	4.2

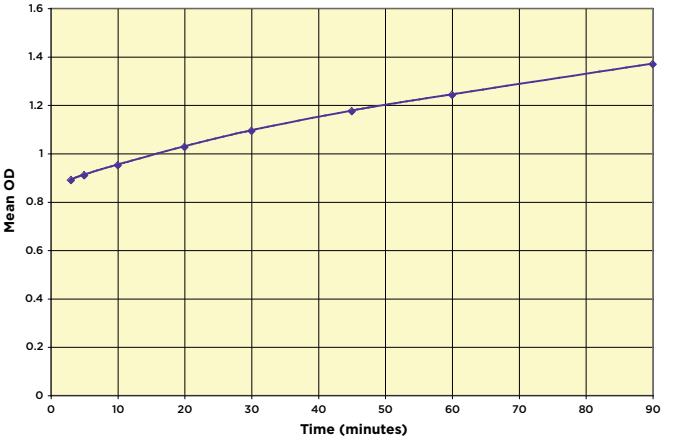
Color Spectrum

The spectra of the FRAP reaction product is shown below.



Kinetics of Color Development

The FRAP assay chemistry is not a true end-point method. Color formation for some samples such as standards and diluted samples is almost instantaneous. Final color for some samples such as serum and plasma continues to develop however the rate of continued color development is relatively slow. The kinetic data below shows room temperature color formation.



LIMITED WARRANTY

Arbor Assays warrants that at the time of shipment this product is free from defects in materials and workmanship. This warranty is in lieu of any other warranty expressed or implied, including but not limited to, any implied warranty of merchantability or fitness for a particular purpose.

We must be notified of any breach of this warranty within 48 hours of receipt of the product. No claim shall be honored if we are not notified within this time period, or if the product has been stored in any way other than outlined in this publication. The sole and exclusive remedy of the customer for any liability based upon this warranty is limited to the replacement of the product, or refund of the invoice price of the goods.

CONTACT INFORMATION

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OFFICIAL SUPPLIER TO ISWE

Arbor Assays and the International Society of Wildlife Endocrinology (ISWE) signed an exclusive agreement for Arbor Assays to supply ISWE members with EIA kits for wildlife conservation research.

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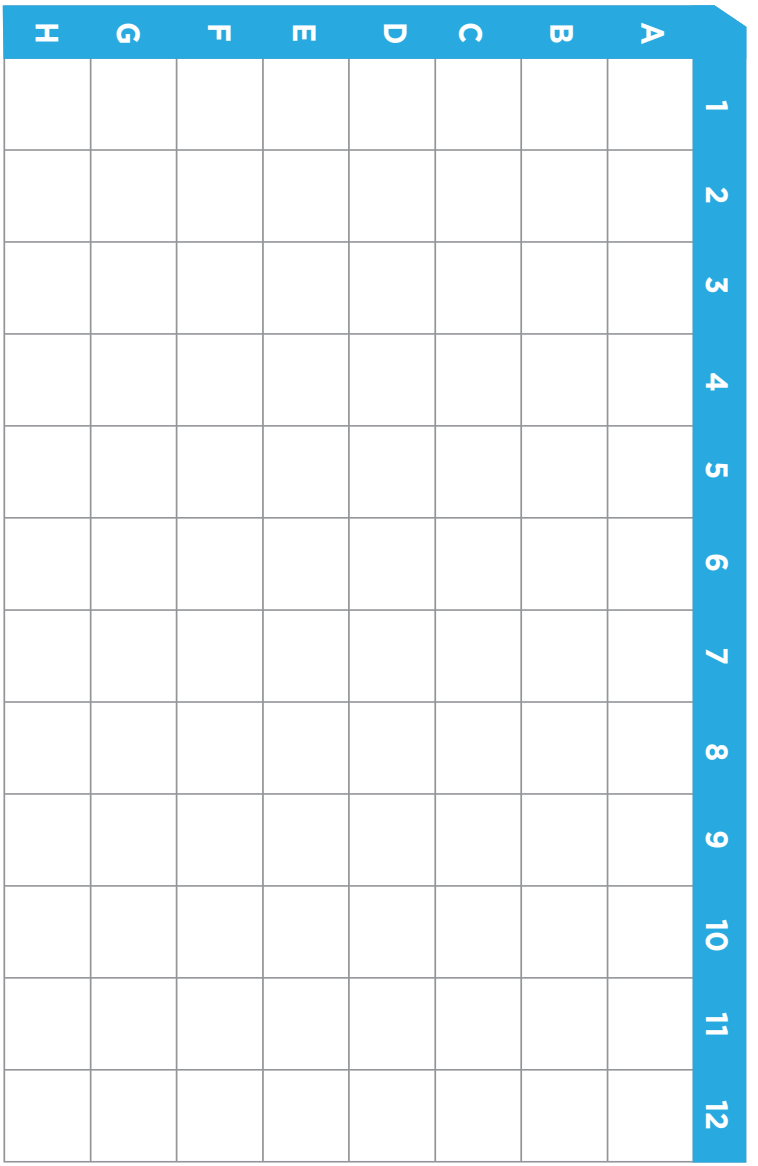
FOR SERIOUS SCIENCE



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