Ferric Antioxidant Status Detection Kit

Catalog Number EIAFECL2 (192 tests)

Rev 1.0

For safety and biohazard guidelines, see the "Safety" appendix in the *ELISA Technical Guide* (Pub. no. MAN0006706). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The Ferric Antioxidant Status Detection Kit is designed to measure the antioxidant status in a variety of samples by gauging the ability of antioxidants to convert ferric ions to ferrous ions.

The assay measures the antioxidant status in serum, plasma (EDTA and heparin), urine, cell lysates, tea, fruit juice, beer, cider, or herbal and fruit extracts. The assay was characterized with human samples, but can be used to test samples from other species.

Reactive oxygen species are produced during normal aerobic metabolism. These free radicals are removed or converted to other products *in vivo* by the activity of specific enzymes (e.g., superoxide dismutase, glutathione peroxidase, catalase, glutathione reductase) as well as nonenzymatic compounds with antioxidant activity (e.g., β-tocopherol, L-ascorbic acid, glutathione, coenzyme Q10, flavonoids, albumin and other molecules).

Contents and storage

Kit and components are shipped at –20°C. Upon receipt, store the kit at –20°C. Once open, store the kit at 4°C and use within 2 weeks.

Components	Quantity
Ferrous Chloride Standard; 10 mM ferrous chloride in a special stabilizing solution	90 µL
Clear 96-well Half Area Plate	2 plates
Assay Buffer Concentrate; 10X acetate buffer with stabilizers and preservatives	25 mL
Ascorbic Acid Control; 100 nmol ascorbic acid	1 vial
FRAP Reagent A	1.4 mL
FRAP Reagent B	1.4 mL

Materials required but not supplied

- Distilled or deionized water
- Microtiter plate reader with software capable of measurement at or near 560 nm
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solution

Procedural guidelines

- Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.
- Solutions containing sodium azide **cannot** be measured using this kit. Azide produces a colored product which interferes with the assay.

Prepare 1X Assay Buffer

1. Dilute 7 mL of Assay Buffer (10X) with 63 mL of deionized or distilled water. Label as 1X Assay Buffer.
2. Store the concentrate and 1X Assay Buffer in the refrigerator. 1X Assay Buffer is stable at 4°C for 3 months.

Sample preparation guidelines

- Collect samples in pyrogen/endotoxin-free tubes.
- Freeze samples after collection if samples will not be tested immediately. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well (do not vortex) prior to analysis.
- Avoid the use of hemolyzed or lipemic sera.
- If large amounts of particulate matter are present in the sample, centrifuge or filter sample prior to analysis.
- Samples containing azide **are not** compatible with the assay.

Dilute samples

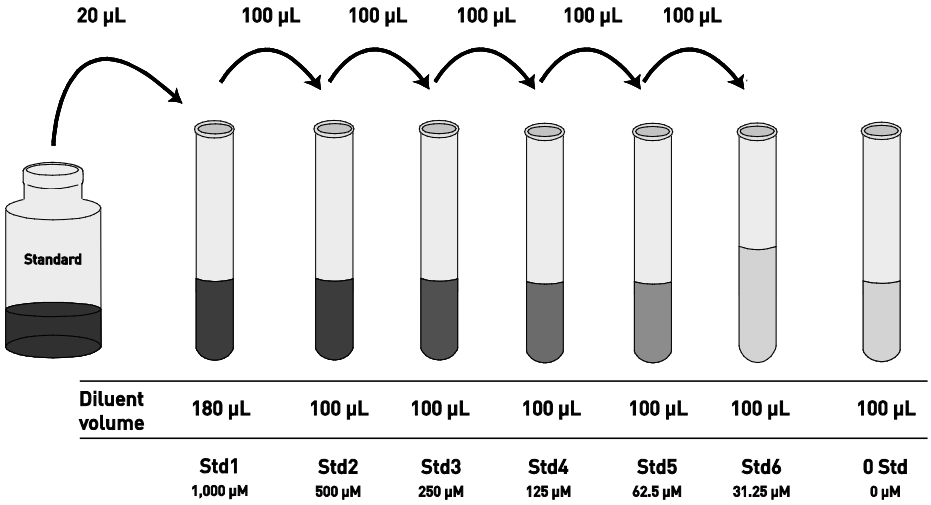
Sample concentrations should be within the range of the standard curve. Because conditions may vary, each investigator should determine the optimal dilution for each application.

- Dilute samples containing ascorbic acid with 1X Assay Buffer to maintain stability.
- Dilute **serum** and **plasma** samples ≥1:2 in 1X Assay Buffer.
- Dilute **urine** samples ≥1:10 in 1X Assay Buffer.
- **Use all samples within 2 hours of dilution.**

Dilute standards

Note: Use glass or plastic tubes for diluting standards.

1. Add 20 µL Ferrous Chloride Standard to one tube containing 180 µL 1X Assay Buffer and label as 1,000 µM $FeCl_2$.
2. Add 100 µL 1X Assay Buffer to each of 6 tubes labeled as follows: 500, 250, 125, 62.5, 31.25, and 0 µM $FeCl_2$.
3. Make serial dilutions of the standard as described below in the dilution diagram. Mix thoroughly between steps.
4. **Use the standards within 2 hours of preparation**.



Reconstitute Ascorbic Acid Control

The Ascorbic Acid Control verifies 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 the 1,000 µM $FeCl_2$ standard.

1. Add 400 µL of 1X Assay Buffer to the vial of Ascorbic Acid Control.
2. Vortex gently for 5 minutes.
3. Store unused reconstituted Ascorbic Acid Control in 50 µL aliquots at –20°C.

Prepare FRAP Color Solution

Add the reagents in the order listed in the following table. **Use the FRAP Color Solution within 2 hours of preparation.**

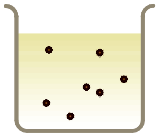
Note: Any significant blue coloration in the FRAP Color Solution may indicate contamination of reagents.

Reagent	½ plate	1 plate	1½ plates	2 plates
1X 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 A	340 µL	625 µL	1 mL	1.25 mL
Total volume	4.08 mL	7.5 mL	12 mL	15 mL

Assay procedure

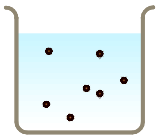
Allow all reagents to reach room temperature before use. Mix all liquid reagents prior to use. **Total assay time is 30 minutes.**

IMPORTANT! Perform a standard curve with each assay.



Add sample

a. Add 20 µL of standards or diluted samples (see page 2) to the appropriate wells.
b. (*Optional*) Add 20 µL of reconstituted Ascorbic Acid Control to positive control wells.



Add color solution

a. Add 75 µL FRAP Color Solution into each well.
b. Incubate for 30 minutes at room temperature.

Read the plate and generate the standard curve

1. Read the absorbance at 560 nm.
2. Use curve-fitting software to generate the standard curve. A four parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.
3. Read the concentrations for unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.

   Note: Dilute samples producing signals greater than that of the highest standard in the appropriate diluent and reanalyze. Multiply the concentration by the appropriate dilution factor.

Performance characteristics

Standard curve (example)

The following data was obtained for the various standards over the range of 0–1,000 µM $FeCl_2$.

Standard $FeCl_2$ (µM)	Optical Density (560 nm)
1,000	2.021
500	1.092
250	0.604
125	0.352
62.5	0.244
31.25	0.177
0	0.116

Intra-assay precision

Three human samples were assayed in replicates of 20 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (µM)	953.0	597.7	236.2
%CV	2.2	2.5	3.0

CV = Coefficient of Variation

Inter-assay precision

Three human samples were assayed 18 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (µM)	951.2	561.2	232.2
%CV	2.9	3.2	4.2

CV = Coefficient of Variation

Performance characteristics, continued

Expected values

This assay was tested with human serum, plasma, urine and with pomeranate juice

Sample	Range (µM)	Average (µM)
Human serum/plasma	700–1000	858.7
Human urine	2547–410	5331
Pomegranate juice	—	82300

Linearity of dilution

Linearity was determined by assaying high and low concentration FRAP level samples mixed in the ratios shown in the following table.

High Sample %	Low Sample %	Expected Conc. (µM)	Observed Conc. (µM)	% Recovery
80	20	94.45	98.59	104.4
60	40	163.40	164.50	100.6
40	60	232.50	234.70	100.9
20	80	301.50	289.80	96.1
			Mean Recovery	**100.5%**

Sensitivity

The analytical sensitivity of the assay is 8.06 µM $FeCl_2$. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

REF Catalog Number | LOT Batch code | Temperature limitation | Use by | Manufacturer | Consult instructions for use | Caution, consult accompanying documents

Manufacturer's address: Life Technologies Corporation | 7335 Executive Way | Frederick, MD 21704 | USA

The information in this guide is subject to change without notice.

DISCLAIMER

TO THE EXTENT ALLOWED BY LAW, LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Important Licensing Information: These products may be covered by one or more Limited Use Label Licenses. By use of these products, you accept the terms and conditions of all applicable Limited Use Label Licenses.

Corporate entity: Life Technologies Corporation | Carlsbad, CA 92008 USA | Toll Free in USA 1 800 955 6288