

## User's Manual and Instructions

### Iron Assay Kit (Z5030022)

#### Quantitative Colorimetric Iron Determination at 590nm

#### DESCRIPTION

Iron level in blood is a reliable diagnostic indicator of various disease states. Increased levels of iron concentration in blood are associated with blood loss, increased destruction of red blood cells (e.g. hemorrhage) or decreased blood cell survival, acute hepatitis, certain sideroachrestic anemias, ingestion of iron-rich diets, defects in iron storage (e.g. pernicious anemia). Decreased levels of blood iron may result from insufficient iron ingestion from diets, chronic blood loss pathologies, or increased demand on iron storage as during normal pregnancy.

Simple, direct and automation-ready procedures for measuring iron concentrations find wide applications in research, drug discovery and environmental monitoring. BioAssay Systems' iron assay kit is designed to measure total iron directly in serum without any pretreatment. The improved method utilizes a chromogen that forms a blue colored complex specifically with  $Fe^{2+}$ .  $Fe^{3+}$  in the sample is reduced to  $Fe^{2+}$ , thus allowing the assay for total iron concentration. The intensity of the color, measured at 590nm, is directly proportional to the iron concentration in the sample.

#### KEY FEATURES

**Sensitive and accurate.** Linear detection range 27  $\mu g/dL$  (4.8  $\mu M$ ) to 1,000  $\mu g/dL$  (179  $\mu M$ ) iron in 96-well plate assay.

**Simple and high-throughput.** The procedure involves addition of a single working reagent and incubation for 40 min. Can be readily automated as a high-throughput assay for thousands of samples per day.

**Improved reagent stability and versatility.** The optimized formulation has greatly enhanced reagent and signal stability. Cuvette or 96-well plate assay.

**Low interference in biological samples.** No pretreatments are needed. Assays can be directly performed on serum samples.

#### APPLICATIONS:

**Direct Assays:** iron in biological samples (e.g. serum).

**Drug Discovery/Pharmacology:** effects of drugs on iron metabolism.

**Environmental Monitoring:** iron in soil extracts, mineralized samples.

#### KIT CONTENTS (250 tests in 96-well plates)

Reagent A: 50 mL Reagent B: 4 mL Reagent C: 4 mL

Iron Standard: 1 mL 10 mg/dL  $Fe^{2+}$

**Storage conditions.** The kit is shipped at room temperature. Store Reagent A at room temperature and other reagents at 4 °C. Shelf life: 12 months after receipt.

**Precautions:** reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

#### PROCEDURES

**Note:** (1). Iron chelators (e.g. EDTA) interfere with this assay and should be avoided in sample preparation. (2). Serum or plasma samples should be clear and free of precipitates or turbidity. If not, centrifuge or filter to clarify samples prior to assay. (3). This kit can be applied to measure  $Fe^{2+}$  (vs. total iron) content. Prepare Working Reagent by mixing 20 vol of Reagent A, 1 vol of water and 1 vol Reagent C (no reductant in the Working Reagent). The procedure is the same as described for the total iron assay.

#### Procedure using 96-well plate:

1. Standards. Prepare 400  $\mu L$  1000  $\mu g/dL$  Premix by mixing 40  $\mu L$  10 mg/dL standard and 360  $\mu L$  distilled water. Dilute standards as follows.

No	Premix + H <sub>2</sub> O	Vol ( $\mu L$ )	Fe ( $\mu g/dL$ )
1	100 $\mu L$ + 0 $\mu L$	100	1000
2	80 $\mu L$ + 20 $\mu L$	100	800
3	60 $\mu L$ + 40 $\mu L$	100	600
4	40 $\mu L$ + 60 $\mu L$	100	400
5	30 $\mu L$ + 70 $\mu L$	100	300
6	20 $\mu L$ + 80 $\mu L$	100	200
7	10 $\mu L$ + 90 $\mu L$	100	100
8	0 $\mu L$ + 100 $\mu L$	100	0

Transfer 50  $\mu L$  diluted standards and 50  $\mu L$  sample into a clear flat bottom 96-well plate. For serum/plasma samples, it is recommended to run a sample blank (i.e. a 50  $\mu L$  sample in a separate well).

2. Prepare enough **Working Reagent** by mixing 20 volumes of Reagent A, 1 volume Reagent B and 1 volume Reagent C. Fresh reconstitution is recommended. Equilibrate to room temperature before assay.

Add 200  $\mu L$  **Working Reagent** to Standards and Samples wells. (For serum/plasma samples which require a Sample Blank Control, add 200  $\mu L$  **Reagent A** to the Sample Blank wells). Tap plate to mix.

3. Incubate 40 min at room temperature and read optical density at 510-630nm (peak absorbance at 590nm).

#### Procedure using cuvette:

1. Prepare standards as in 96-well assay. Set up centrifuge tubes labeled Standards and Samples. Transfer 250  $\mu L$  standards and samples to tubes.

2. Add 1000  $\mu L$  **Working Reagent** to all tubes. Mix by vortexing. Incubate 40 min at room temperature.

3. Transfer to cuvettes and read OD at 590nm (510nm-630nm).

#### CALCULATION

Subtract OD of "0  $\mu g/dL$  Fe" from all other standard OD values and plot the OD against standard iron concentrations. Determine the slope using linear regression fitting. Iron concentration of the sample is calculated as

$$[\text{Iron}] = \frac{OD_{\text{SAMPLE}} - OD_{\text{BLANK}}}{\text{Slope}} \quad (\mu g/dL)$$

Where  $OD_{\text{BLANK}}$  is OD values of the water blank (Standard #8), or Sample Blank, if a sample blank is used (e.g. serum or plasma). Typical serum iron values: 70-180  $\mu g/dL$ .

**Conversions:** 1 mg/dL Fe equals 179  $\mu M$ , 0.001% or 10 ppm.

#### MATERIALS REQUIRED, BUT NOT PROVIDED

Pipeting devices and accessories.

#### Procedure using 96-well plate:

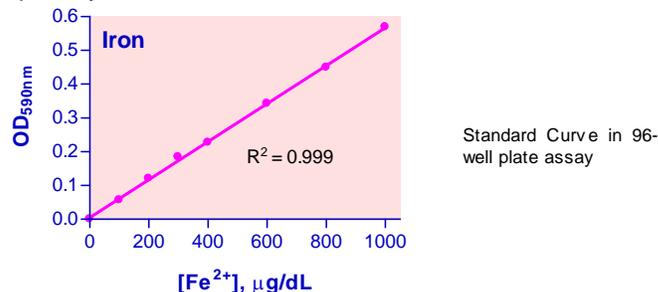
Clear bottom 96-well plates (e.g. Corning Costar) and plate reader.

#### Procedure using cuvette:

Cuvets and spectrophotometer for measuring OD at 510-630nm.

#### EXAMPLES:

Mouse serum, fetal bovine serum (Invitrogen), and goat serum (Invitrogen) were assayed using the 96-well plate assay protocol. The iron concentrations were  $173 \pm 2$  (n = 4),  $149 \pm 1$  (n = 4),  $88 \pm 2$   $\mu g/dL$  (n = 4), respectively. Coefficient of variance < 2%.



#### PUBLICATIONS

- Habel, M-E. and Jung, D. (2006). c-Myc over-expression in Ramos Burkitt's lymphoma cell line predisposes to iron homeostasis disruption in vitro. *Biochem. Biophys Res Comm* 341(4):1309-1316.
- Wu, Y. J. et al (2007). In vivo leukocyte labeling with intravenous ferumoxides/protamine sulfate complex and in vitro characterization for cellular magnetic resonance imaging. *Am J Physiol Cell Physiol* 293: C1698-C1708.
- Raulfs, E. C. et al (2008). *In vivo* iron-sulfur cluster formation. *PNAS* 105:8591-8596.