Iron Assay Kit

ARG83671 Iron Assay Kit is a detection kit for the quantification of Iron levels in a variety of samples.

Catalog number: ARG83671

Package: 96 wells

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MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: 9F.-7, No. 12, Taiyuan 2nd St., Zhubei City,

Hsinchu County 302082, Taiwan

Tel: +886-3-6221320

Fax: +886-3-5530266

Email: info@arigobio.com

PRINCIPLE OF THE ASSAY

Iron Microplate Assay Kit provides a simple and direct procedure for measuring iron levels in a variety of samples. The ferrium ions can react with Phenanthroline. The intensity of the color is measured at a wavelength of 510 nm ±2nm. The concentration of ferrium ions in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8°C. Use the kit before expiration date.

Component	Quantity	Storage
Microplate	1 X 96-well plate	RT
Standard (500 nmol/mL)	1 mL	4°C
Assay Buffer I	4 X 30 ml (ready to use)	4°C
Reducing Reagent	1 vial (lyophilized)	4°C
Reaction Buffer	5 ml (ready to use)	4°C
Reagent Dye	1 vial (lyophilized)	4°C
Plate sealer	4 strips	Plate sealer

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 510 nm
- Pipettes and pipette tips
- Deionized or distilled water

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the unopened kit at 2-8°C. Use the kit before expiration date.
- Briefly spin down the reagents before use.
- It is highly recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum or plasma-add 0.5 ml Assay Buffer into 0.5 ml sample, mix, centrifuged at 10000g for 10 minutes, take the supernatant into a new centrifuge tube for detection.

Cell and bacteria samples- Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 0.5 ml ddH2O for 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); then add 0.5 ml Assay Buffer mix, centrifuged at 10000g for 10 minutes, take the supernatant into a new centrifuge tube for detection.

Tissue samples- Weigh out 0.1 g tissue, homogenize with 0.5 ml ddH2O, then add 0.5 ml Assay Buffer mix, centrifuged at 10000g for 10 minutes, take the supernatant into a new centrifuge tube for detection.

Liquid sample- Liquid samples can be tested directly.

REAGENT PREPARATION

- **Standard:** Perform 2-fold serial dilution of the top standards to make the standard curve.
- **Sample**: If the measuring absorbance of samples is higher than the standard, dilute the samples with **Distilled water** before assay and assay again. For the calculation of the activity this dilution factor has to be taken into account.
- **Reducing Reagent:** Reconstitute the **Reducing Reagent** with **2.5 ml** of distilled water. Allow the **Reducing Reagent** keep on bench for few minutes. Make sure the **Reducing Reagent** is dissolved completely and mixed thoroughly before use.
- **Reagent Dye:** Reconstitute the **Reagent Dye** with **2.5 ml** of distilled water. Allow the **Reagent Dye** keep on bench for few minutes. Make sure the **Reagent Dye** is dissolved completely and mixed thoroughly before use.

ASSAY PROCEDURE

Standards and samples should be assayed in at least duplicates.

1. Sample wells: Add **100 µl Sample** into Sample wells.
2. Standard wells: Add **100 µl Standard** into Standard wells.
3. Add **25 µl** of **Reducing Reagent** to each wells.
4. Add **50 µl** of **Reaction Buffer** to each wells.
5. Add **25 µl Reagent Dye** to each wells.
6. Mix well. Incubate at **RT** for **2 min**. Read the OD at **510 nm**

Reagent	Sample	Standard	Blank
Sample	100 µl	-	-
Standard	-	100 µl	-
Distilled water	-	-	100 µl
Reducing Reagent	25 µl	25 µl	25 µl
Reaction Buffer	50 µl	50 µl	50 µl
Reagent Dye	25 µl	25 µl	25 µl
Mix well. Incubate at 37°C for 60 min. Read the OD at 510 nm			

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of samples, standard and blank.

2. Calculation:

A. Definition:

$C_{Standard}$: the standard concentration, 500 nmol/mL= 0.5 µmol/ml;

W: the weight of sample, g;

V_{Sample}: the volume of reaction sample, 100 µl = 0.1 ml;

$V_{standard}$: the volume of standard sample, 100 µl = 0.1 ml;

N: the quantity of cell or bacteria, $N \times 10^4$;

V_{assay}: the volume of ddH2O + Assay Buffe, 1 ml.

B. Formula:

a). According to the volume of sample:

Iron (µmol /mL) =

$2X\ [(C_{Standard} \times V_{standard}) \times (OD_{Sample}) - OD_{Blank})] / [(OD_{Standard} - OD_{Blank}) \times V_{Sample}]$

$= (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$

b). According to the weight of sample:

Iron (µmol /g) =

$[(C_{Standard} \times V_{standard}) \times (OD_{Sample}) - OD_{Blank})] / [(OD_{Standard} - OD_{Blank}) X (W \times V_{Sample} / V_{Assay})]$

$= 0.5x\ (OD_{Sample} - OD_{Blank}) / [(OD_{Standard} - OD_{Blank}) \times W]$

c). According to the quantity of cells or bacteria:

Iron (μmol /10^4) =

$[(C_{Standard} \times V_{standard}) \times (OD_{Sample}) - OD_{Blank})] / [(OD_{Standard} - OD_{Blank}) \times (N \times V_{Sample} / V_{Assay}]$

$= 0.5x\ (OD_{Sample} - OD_{Blank}) / [(OD_{Standard} - OD_{Blank}) \times N]$

3. Detection range:

The detection range is from 5- 500 nmol/mL.

4. If the samples have been diluted, the calculated activity must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL RESULT

The following data is for demonstration only and cannot be used in place of data generations at the time of assay. Please note this data is for demonstration only and serial diluted standards are not necessary for this kit.