# ab83366 – Iron Assay Kit (Colorimetric)

For rapid, sensitive and accurate measurement of ferrous and/or ferric levels in various samples.
For research use only - not intended for diagnostic use.

**For overview, typical data and additional information please visit:**
**http://www.abcam.com/ab83366** (use http://www.abcam.cn/ab83366 for China, or http://www.abcam.co.jp/ab83366 for Japan)

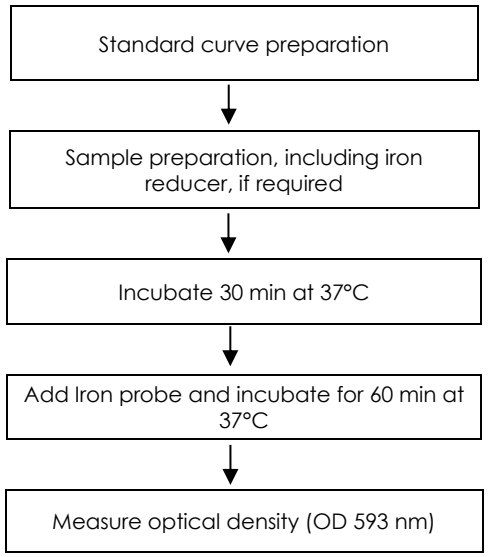
**Background:**

Iron Assay Kit ab83366 provides a simple, rapid, and sensitive method for measuring ferrous ($Fe^{2+}$) and/or ferric ($Fe^{3+}$) iron in biological samples, such as serum, heparin plasma, cells and tissue lysates.

Ferric carrier protein will dissociate ferric iron into solution in the presence of the acid assay buffer (pH < 5.5). After reduction of the free ferric ($Fe^{3+}$) to ferrous iron ($Fe^{2+}$), which will react with the Iron Probe to produce a stable chromophore detectable by absorbance at 593 nm. Ferrous iron ($Fe^{2+}$) can be measured alone, or by addition of the reducing agent total iron ($Fe^{2+}$ and $Fe^{3+}$) can be measured. The amount of ferric iron ($Fe^{3+}$) is calculated by subtracting ferrous iron from total iron. A specific chelate chemical is included in the buffer to block copper ion ($Cu^{2+}$) interference. The kit measures iron in the linear range of 0.4 to 10 nmol or 8 µM to 200 µM.

**Assay Summary:**

NOTE: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.




## QUICK ASSAY PROCEDURE

- Set incubator to 37°C
- Thaw all kit components and get equipment ready.
- Prepare samples in duplicate (find optimal dilutions to fit standard curve readings).
- Prepare standard curve.
- Set up plate for standard (100 µL) and samples (100 µL).
- If measuring total iron, add 5 µL Iron Reducer to corresponding samples.
- Incubate for 30 min at 37°C.
- Add 100 µL Iron Probe to each well.
- Incubate plate for 60 mins or at 37°C.

**Precautions & Limitations:**

**Please read these instructions carefully prior to beginning the assay.**
All kit components have been formulated and quality control tested to function successfully as a kit.

- Modifications to the kit components or procedures may result in loss of performance.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

**Storage and Stability**:

Store kit at -20°C in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted. Reconstituted components are stable for 2 months. Do not use kit or components if they have exceeded the expiry date.

**Materials Supplied:**

| Item | Quantity | Storage Temperature (on receipt) | Storage temperature (reconstituted) |
|---|---|---|---|
| Iron Assay Buffer | 25 mL | -20°C | -20°C |
| Iron Probe | 12 mL | -20°C | -20°C |
| Iron Reducer | 700 µL | -20°C | -20°C |
| Iron Standard I | 100 µL | -20°C | -20°C |

**Materials Required, Not Supplied:**

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance (OD) at 593 nm
- 96 well clear plate with clear flat bottom
- Dounce homogenizer (if using tissues or cells)
- Microcentrifuge
- 1 x PBS pH 7.4
- MilliQ water or other type of double distilled/deionized water (dd$H_2O$)

**Reagent Preparation:**

- Briefly centrifuge small vials at low speed prior to opening.
- Equilibrate reagents to room temperature before use.
- Aliquot reagents so that you have enough volume to perform the desired number of assays.

**Iron Standard I** and **Iron Reducer** are ready to use as supplied. Equilibrate to room temperature before use. Mix the iron reduced to dissolve any precipitate that may have formed during freezing. Store at -20°C and protect from light and moisture.

**Iron Probe:** Ready to use as supplied. Keep on ice during the assay. Equilibrate to room temperature before use. Store at -20°C and **protect from light and moisture**. Once the probe is opened and thawed, it is stable for at least 3 additional freeze/thaw cycles but should be used within two months. After use, promptly retighten the cap to minimize adsorption of airborne moisture.

**Iron Assay Buffer** is ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C and protect from light and moisture.

**Sample Preparation:**

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step before storing the samples at -20°C. Alternatively, snap freeze your samples in liquid nitrogen upon extraction and store them immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples, and the readings can be lower than expected.

- Interferences:
  - This assay is **not** suitable for use with plasma samples containing EDTA or citrate. Chelation anticoagulants such as EDTA and Citrate bind to Iron and will interfere with the assay (heparinized plasma or off-the-clot serum are recommended).
  - The following chemicals or biological materials will cause interference in this assay resulting in compromised results: Chromium (III), Copper (II) and Transferrin.

**Plasma Samples**:

1. The preferred sample is off-the-clot serum; however, if plasma is used, sodium or lithium heparin is the only compatible anticoagulant.
2. Heparin plasma can be used directly. Up to 50 µl of plasma can be added to the wells.

**Serum (off-the-clot) Samples:**

1. Collect whole blood in a covered test tube on ice.
2. After collection of the whole blood, allow the blood to clot by leaving it undisturbed at room temperature (typically 15 – 30 minutes).
3. Collect supernatant and transfer to a clean tube Remove the clot by centrifuging samples at 1000 – 2000 x g for 10 minutes in a cold microcentrifuge.
4. Following centrifugation, it is important to immediately transfer the serum into a clean polypropylene tube using a Pasteur pipette.
5. Off-the-clot can be used directly. Up to 50 µl of serum can be added to the wells
6. If samples are not analyzed immediately, serum should be aliquoted into 0.5 mL aliquots. Store at -80°C.
   **Note**: Normal serum iron concentration ~ 10 – 40 µM.

**Cells (adherent or suspension) samples:**

1. Harvest the number of cells necessary for each assay (initial recommendation = 5 x $10^6$ cells/well).
2. Wash cells in cold PBS.
3. Pellet cells in a tube by spinning at 2,000 rpm for 5 minutes, and discard supernatant.
4. Lyse cells with 500 µL of Iron Assay Buffer by homogenization with a Dounce homogenizer sitting on ice.
5. Vortex the extraction for 10 seconds.
6. Centrifuge for 10 minutes at 4°C at top speed using a cold microcentrifuge to remove any insoluble material.
7. Collect supernatant and transfer into a new tube. Keep on ice.

**Tissue Samples:**

1. Harvest the amount of tissue necessary for each assay (initial recommendation = 100 mg).
2. Wash tissue in cold PBS.
3. Homogenize in 500 µL Iron Assay Buffer using a Dounce homogenizer (30 – 50 passages).
4. Centrifuge 10 minutes at 4°C at top speed in a cold microcentrifuge to remove any insoluble material.
5. Collect supernatant into a new tube. Keep on ice.

**Other biological fluids:**

– To be used directly in the assay without sample preparation. Perform dilutions if needed.

**Standard Preparation:**

- Always prepare a fresh set of standards for every use.
- Diluted standard solution is unstable and must be used within 4 hours.
- Each dilution has enough standard to set up duplicate readings (2 x 100 µL).

Prepare 1 mM Iron Standard I as follows:
Add 10 µL of undiluted Iron Standard I to 990 µL $ddH_2O$. Gently pipette up and down a few times to ensure all standard is removed from tip. Mix well by inversion.

Using 1 mM Iron standard I, prepare standard curve dilution as described in the table below in a microplate or microcentrifuge tubes:

| Standard # | Volume of 1 mM Standard (µL) | Iron Reducer (µL) | Assay Buffer (µL) | Final volume standard in well (µL) | End Iron Amount (nmoles/well) |
|---|---|---|---|---|---|
| 1 | 0 | 15 | 285 | 100 | 0 |
| 2 | 6 | 15 | 279 | 100 | 2 |
| 3 | 12 | 15 | 273 | 100 | 4 |
| 4 | 18 | 15 | 267 | 100 | 6 |
| 5 | 24 | 15 | 261 | 100 | 8 |
| 6 | 30 | 15 | 255 | 100 | 10 |

**Assay Procedure:**

- Equilibrate all other materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls, and samples in duplicate.

1. Set up Reaction wells (see table below):
   - Standard wells = 100 µL standard dilutions.
   - Sample wells = 2 – 50 µL samples (adjust volume to 100 µL/well with Iron Assay Buffer).
2. Add 5 µL Iron Reducer to each Standard well.
3. For Ferrous Iron ($Fe^{2+}$) assay: add 5 µL of Assay Buffer to each sample.
4. For Total Iron ($Fe^{2+}$ -and $F^{3+}$) assay: add 5 µL of Iron Reducer to each sample.
5. Mix and incubate standards and samples at 37°C for 30 minutes.
6. Add 100 µL Iron Probe to each well containing the Iron Standard I and test samples.
7. Mix and incubate at 37°C for 60 minutes protected from light.
8. Measure output immediately on a microplate reader at OD 593 nm for colorimetric assay.

| Component Order | Standard (µL) | Ferrous Iron (Fe ) (µL) | Total Iron (Fe and Fe ) (µL) |
|---|---|---|---|
| 1. Standard | 100 | - | - |
| 2. Sample | - | 100 | 100 |
| 3. Iron Reducer | 5 | - | 5 |
| 4. Iron Buffer | - | 5 | - |
| 5. Iron Probe | 100 | 100 | 100 |

**Calculations:**

- For samples producing signals greater than that of the highest standard: dilute further in appropriate buffer and reanalyze. Multiply the concentration found by the appropriate dilution factor.

1. Average the duplicate reading for each standard and sample.
2. Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.
3. Plot the corrected absorbance values for each standard as a function of the final amount of Iron per well. Calculate the best-fit equation of the standard curve using a linear regression and determine the slope.
4. Interpolate the amount (in nmoles) of Ferrous Iron ($Fe^{2+}$) or Total Iron ($Fe^{2+}$ and $Fe^{3+}$) (*B*) in the sample wells by using the linear equation, using the sample readings for Ferrous Iron and Total Iron sample wells.
5. Ferric Iron ($Fe^{3+}$) content of the test sample can be calculated as:

Ferric Iron ($Fe^{3+}$) = Total Iron ($Fe^{2+}$ and $Fe^{3+}$) – Ferrous Iron ($Fe^{2+}$)

6. Concentrations of Ferrous, Ferric or Total Iron in the test samples is calculated as:

$$Iron\ Concentration = \left(\frac{B}{V}\right) \times D = nmoles/\mu L = mM$$

Where:
$B$ = calculated amount of corresponding Iron (ferrous, ferric or total) in the sample well (in nmoles).
$V$ = volume of sample added to the sample wells (in µL).
$D$ = sample dilution factor ($D$ = 1 for neat plasma/serum samples).

**Technical Hints**

For additional helpful hints and tips on using our assay kits please visit:
https://www.abcam.com/en-us/support/product-support

**Technical Support**



**For technical or commercial enquiries please go to:**

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