

Rat Ferritin ELISA Kit

Catalog No.: abx051446

Size: 96T

Range: 1.6 ng/ml - 150 ng/ml

Sensitivity: 0.5 ng/ml

Storage: Store at 2-8°C for 6 months.

Application: For quantitative detection of Ferritin in Rat serum, plasma, cell culture supernatant or any biological fluid.

Introduction

Ferritin is a ubiquitous intracellular protein that stores iron and releases it in a controlled fashion. The protein is produced by almost all living organisms, including algae, bacteria, higher plants, and animals. In humans, it acts as a buffer against iron deficiency and iron overload. Ferritin is found in most tissues as a cytosolic protein, but small amounts are secreted into the serum where it functions as an iron carrier. Plasma ferritin is also an indirect marker of the total amount of iron stored in the body, hence serum ferritin is used as a diagnostic test for iron deficiency anemia. Ferritin is a globular protein complex consisting of 24 protein subunits and is the primary intracellular iron-storage protein in both prokaryotes and eukaryotes, keeping iron in a soluble and non-toxic form. Ferritin that is not combined with iron is called apoferritin.

Principle of the Assay

This kit is based on sandwich enzyme-linked immunosorbent assay technology. Anti-Ferritin antibody is pre-coated onto 96-well plates. An HRP conjugated anti-Ferritin antibody is used as detection antibody. The standards, test samples and HRP conjugated detection antibody are added to the wells, incubated. Unbound conjugates are washed away with wash buffer. TMB substrate is used to visualize HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue color product that changes into yellow after adding acidic stop solution. The density of yellow is proportional to the Ferritin amount of sample

captured in plate. The O.D. absorbance is measured spectrophotometrically at 450nm in a microplate reader, and then the concentration of Ferritin can be calculated.

Kit components

1. One 96-well plate pre-coated with anti-Rat Ferritin antibody
2. Rat Ferritin Standard: 0.5 ml
3. Standard diluent buffer: 1.5 ml
4. Wash buffer (30x): 20 ml. Dilution: 1:30
5. Sample diluent buffer: 6 ml
6. HRP conjugated anti-Rat Ferritin antibody (RTU): 6ml
7. Stop solution: 6 ml
8. TMB substrate A: 6ml
9. TMB substrate B: 6ml
10. Plate sealer: 2
11. Hermetic bag: 1

Material Required But Not Provided

1. 37°C incubator
2. Microplate reader (wavelength: 450nm)
3. Precision pipette and disposable pipette tips
4. Automated plate washer
5. ELISA shaker
6. 1.5ml tubes to prepare standard/sample dilutions.
7. Plate cover
8. Absorbent filter papers
9. 100 ml and 1 L volume graduated cylinder.

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Protocol

A. Preparation of sample and reagents

1. Sample

Isolate the test samples soon after collecting and analyze immediately (within 2 hours) or aliquot and store at -20°C or -80°C for long term storage. Avoid multiple freeze-thaw cycles.

✧ **Cell culture supernatant:** Centrifuge at approximately 2000-3000 × g for 20 min to remove precipitant. Analyze immediately or aliquot and store at -20°C or -80°C.

✧ **Serum:** Samples should be collected into a serum separator tube. Incubate at room temperature (~ 2 hour) or overnight at 4°C. Centrifuge at ~ 2000-3000 × g for 10 - 20 min. Analyze immediately or aliquot and store at -20°C or -80°C.

✧ **Plasma:** Collect plasma with citrate or EDTA as the anticoagulant. Mix for 20 min approximately. Centrifuge at 2000-3000 × g for 20 min within 30 min of collection. Analyze immediately or aliquot and store at -20°C or -80°C.

✧ **Urine:** Collect in a sterile container. Centrifuge at 10,000 × g for 2 min at 4°C.

✧ **Tissues:** The preparation of tissue homogenates will vary depending upon tissue type. Rinse tissues with ice-cold PBS to remove the excess of blood. Weigh before homogenization. Finely mince tissues and homogenize in PBS with a tissue homogenizer on ice. Homogenate completely and centrifuge to collect the supernatant. Analyze immediately or aliquot and store at -20°C or -80°C.

Note:

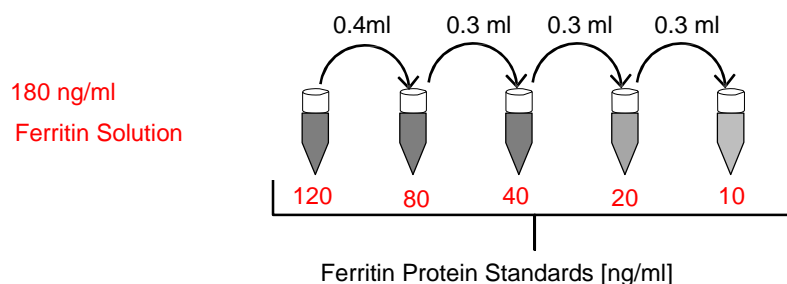
- Samples must be diluted so that the expected concentration falls within the kit's range.
- Fresh samples or recently obtained samples are recommended to prevent protein degradation and denaturalization that may lead to erroneous results.
- Coagulate blood samples completely, centrifuge, and avoid hemolysis and precipitant. Hemolysis will influence the result. Please bring samples slowly to room temperature.
- NaN₃ cannot be used as test sample preservative, since it inhibits HRP.

2. Wash buffer

Dilute the concentrated Wash buffer 30-fold (1/30) with distilled water (i.e. add 20 ml of concentrated wash buffer into 580 ml of distilled water).

3. Standard

Label 5 tubes with 120 ng/ml, 80 ng/ml, 40 ng/ml, 20 ng/ml and 10 ng/ml respectively. Aliquot **0.2 ml** of the Standard diluent buffer into the first two tubes labeled 120 ng/ml and 80 ng/ml respectively and **0.3 ml** of the Standard diluent buffer into each remaining tube. Add **0.4 ml** of 180 ng/ml standard solution into 1st tube and mix thoroughly. Transfer **0.4 ml** from 1st tube to 2nd tube and mix thoroughly. Transfer **0.3 ml** from 2nd tube to 3rd tube and mix thoroughly, and so on. The standard solutions are best used within 2 hours. Avoid repeated freeze-thaw cycles.



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B. Assay Procedure

Equilibrate the kit components and samples to room temperature prior to use. It is recommended to plot a standard curve for each test.

1. Set standard, test sample and control (zero) wells on the pre-coated plate respectively, record their positions. It is recommended to assay standards, samples and controls in duplicate.
2. Aliquot 50 µl of the diluted standards into the standard wells.
3. Aliquot 50 µl of Standard diluent buffer into the control (zero) well. Do not add sample and HRP conjugated antibody into the control (zero) well.
4. Aliquot 50 µl of appropriately diluted sample (Rat serum, plasma or cell culture supernatants) into the test sample wells. Add the solution at the bottom of each well without touching the side wall. Shake the plate mildly to mix the contents.
5. Seal the plate with a cover and incubate at 37°C for 30 min.
6. Remove the cover and wash the plate 5 times with one of the following methods.

Manual Washing: Discard the solution without touching the side walls. Tap the plate on absorbent paper or other absorbent material. Fill each well completely with wash buffer and vortex mildly on ELISA shaker for 2 min. Discard the contents and tap the plate on absorbent filter papers or other absorbent material. Repeat this procedure five times.

Automated Washing: Discard the solution in all wells and wash the plate five times with Wash buffer (overfilling the wells with the buffer). After the final wash invert the plate and tap on absorbent filter papers or other absorbent material. It is recommended that the washer be set for a soaking time of 1 min.

7. Aliquot 50 µl of HRP conjugated anti-Ferritin antibody into each well (except control well).
8. Seal the plate with a cover and incubate at 37°C for 30 min.
9. Remove the cover and repeat the aspiration/wash process 5 times as explained in step 6.
10. Aliquot 50 µl of TMB Substrate A into each well and 50 µl of TMB Substrate B. Vortex gently the plate on ELISA shaker for 30 seconds (Or shake gently by hand for 30 seconds). Cover the plate and incubate at 37°C for 15 min. Avoid exposure to light.
11. Aliquot 50 µl of Stop solution into each well and mix thoroughly. The color should change to yellow immediately.
12. Read the O.D. absorbance at 450 nm in a microplate reader within 15 min of adding the stop solution.

For calculation, (the relative O.D.₄₅₀) = (the O.D.₄₅₀ of each well) – (the O.D.₄₅₀ of Zero well). The standard curve can be plotted as the relative O.D.₄₅₀ of each standard solution (Y) vs. the respective concentration of the standard solution (X). The Rat Ferritin concentration of the samples can be interpolated from the standard curve.

Note: If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

C. Precautions

1. Before using the kit, centrifuge the tubes briefly to bring down the contents trapped in the lid.
2. Avoid foaming or bubbles when mixing or reconstituting components.
3. Wash buffer may crystallize and separate. If this happens, please warm the tube and mix gently to dissolve.
4. It is recommend to measure each standard and sample in duplicate or triplicate.
5. Do NOT let the plate completely dry at any time! This can inactivate the biological material on the plate.
6. To avoid cross contamination do not reuse pipette tips and tubes.

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7. Do not use expired components or components from a different kit.
8. Store the TMB substrate B in the dark and to avoid edge effect of plate incubation for temperature differences it is recommended to equilibrate the TMB substrates for 30 min at room temperature prior to use. Aspirate the dosage needed with sterilized tips and do not dump the residual solution back into the vial.

D. Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level Rat Ferritin were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, middle and high Rat Ferritin were tested on 3 different plates, 8 replicates in each plate.

$$CV (\%) = SD/\text{mean} \times 100$$

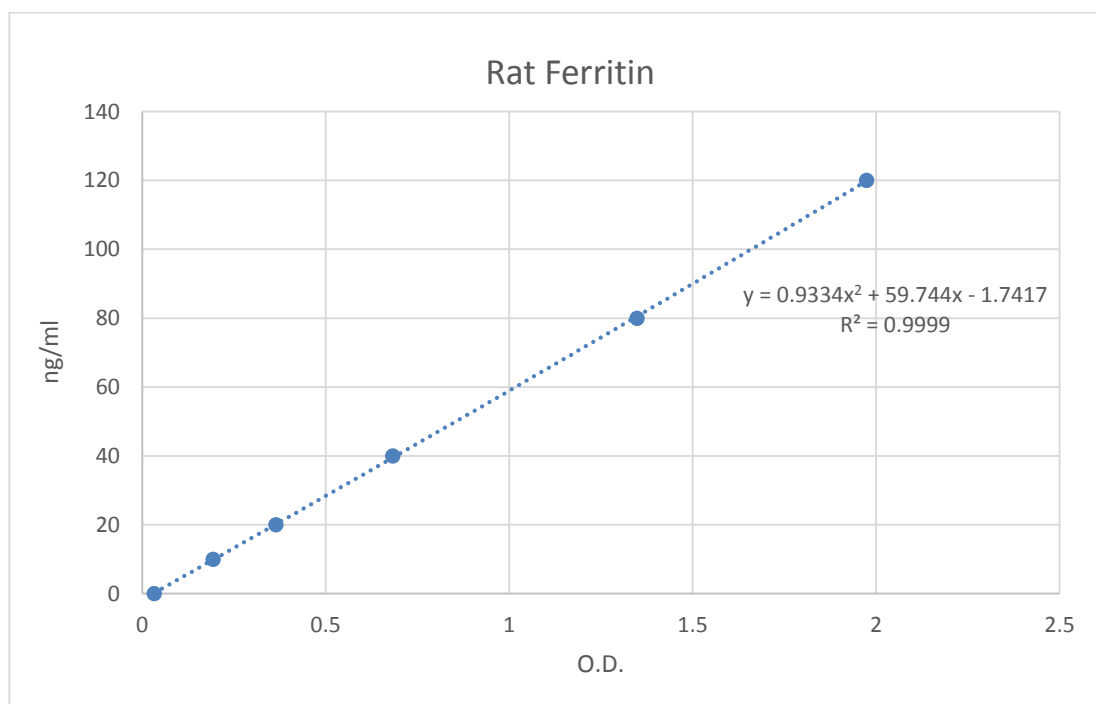
Intra-Assay: CV < 10%

Inter-Assay: CV < 12%

E. Typical Data & Standard Curve

Results of a typical standard run of a Rat Ferritin ELISA Kit are shown below. This standard curve was generated at our lab for demonstration purpose only. Each user should obtain their own standard curve as per experiment. (N/A=not applicable)

| ng/ml | 0 | 10 | 20 | 40 | 80 | 120 |
|-------|-------|-------|-------|-------|-------|-------|
| OD450 | 0.033 | 0.193 | 0.364 | 0.683 | 1.348 | 1.974 |



This diagram is for reference only