KAMIYA BIOMEDICAL COMPANY

Rat Ferritin ELISA

For the quantitative determination of ferritin in rat biological samples

Cat. No. KT-413

For research use only.

PRODUCT INFORMATION

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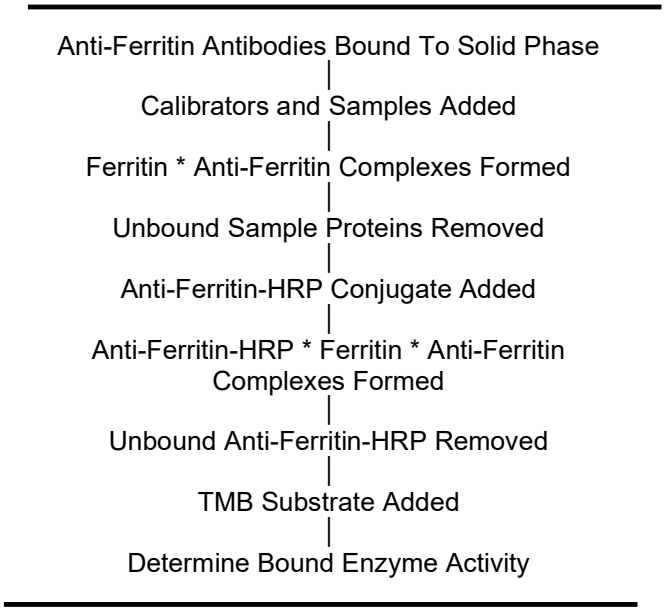
INTENDED USE
The Rat Ferritin ELISA is a highly sensitive two-site enzyme-linked immunoassay (ELISA) for the quantitative determination of ferritin in rat biological samples. For research use only.

INTRODUCTION
Ferritin is a water-soluble, iron storage protein. Serum ferritin level is said to be useful for studying iron deficiency anemia, metabolism disorder and malignant tumor. Ferritin may also be an acute-phase protein and is often elevated in the course of disease.

PRINCIPLE
The principle of the double antibody sandwich ELISA is represented in Figure 1. In this assay the ferritin present in the sample reacts with the anti-ferritin antibodies which have been adsorbed to the surface of polystyrene microtiter wells. After the removal of unbound proteins by washing, anti-ferritin antibodies conjugated with horseradish peroxidase (HRP) are added. This HRP-conjugated antibody forms a complex with the previously bound ferritin. Following another washing step, the enzyme bound to the immunosorbent is assayed by the addition of a chromogenic substrate, 3,3',5,5'-tetramethylbenzidine (TMB). The quantity of bound enzyme is proportional to the concentration of ferritin in the sample tested; thus, the absorbance, at 450 nm, is a measure of the concentration of ferritin in the test sample. The quantity of ferritin in the test sample can be interpolated from the calibration curve constructed from the calibrators, and corrected for sample dilution.

Figure 1.



COMPONENTS

1. Diluent Concentrate
   One bottle containing 50 mL of a 5X concentrated diluent running buffer.

2. Wash Solution Concentrate
   One bottle containing 50 mL of a 20X concentrated wash solution.

3. Enzyme-Antibody Conjugate Concentrate
   One vial containing 150 μL of a 100X concentrated affinity-purified anti-rat ferritin antibody conjugated with HRP in stabilizing buffer.

4. TMB Substrate Solution
   One bottle containing 12 mL of TMB and hydrogen peroxide in citric acid buffer at pH 3.3.

5. Stop Solution
   One bottle containing 12 mL of 0.3 M sulfuric acid.
   WARNING: Avoid contact with skin.

6. Microtiter Plate
   Twelve removable eight-well micro strips in well holder frame. Wells are coated with affinity-purified anti-rat ferritin.

7. Rat Ferritin Calibrator
   One vial containing a lyophilized Rat Ferritin Calibrator.

8. Positive Control
   One vial containing 50 μL of serum with 0.1% sodium azide. See the Control Certificate for the concentration.

MATERIALS REQUIRED BUT NOT PROVIDED

- Precision pipettes (2 μL to 200 μL) for making and dispensing dilutions
- Test tubes
- Squirt bottle or Microplate washer/aspirator
- Distilled or de-ionized H_2O
- Microplate reader
- Assorted glassware for the preparation of reagents and buffer solutions
- Timer
- Centrifuge for sample collection
- Anticoagulant for plasma collection

PRECAUTIONS

1. Read the instructions carefully before beginning the assay.
2. This kit is for research use only.
3. Great care has been taken to ensure the quality and reliability of this product. However, it is possible that in certain cases, unusual results may be obtained due to high levels of interfering factors.
4. Preservatives
   - Positive Control contains 0.1% sodium azide.
5. Azide and thimerosal at concentrations higher than 0.1% inhibit the enzyme reaction.
6. Other precautions:
   - Do not interchange kit components from different lots.
   - Do not use kit components beyond the expiration date.
   - Protect reagents from direct sunlight.
   - Do not pipette by mouth.
   - Do not eat, drink, smoke or apply cosmetics where reagents are used.
   - Avoid all contact with the reagents by using gloves.
   - Stop solution contains diluted sulfuric acid. Irritation to eyes and skin is possible. Flush with water after contact.

REAGENT PREPARATION

1. Diluent Concentrate
   The Diluent solution supplied is a 5X concentrate and must be diluted 1:5 with distilled or de-ionized water.

2. Wash Solution Concentrate
   The Wash Solution supplied is a 20X concentrate and must be diluted 1:20 with distilled or de-ionized water. Crystal formation in the concentrate is not uncommon when storage temperatures are low. Warming of the concentrate to 30-35°C before dilution can dissolve crystals.

3. Enzyme-Antibody Conjugate Concentrate
   Calculate the required amount of working conjugate solution for each microtiter plate test strip by adding 10 µL Enzyme-Antibody Conjugate to 990 µL of 1X Diluent for each test trip to be used for testing. Mix uniformly, but gently. Avoid foaming. Dilute immediately before use and protect from light.

4. TMB Substrate Solution
   Ready to use as supplied.

5. Stop Solution
   Ready to use as supplied.

6. Microtiter Plate
   Ready to use as supplied. Unseal microtiter pouch and remove plate from pouch. Remove all strips and wells that will not be used in the assay and place back in pouch and re-seal along with desiccant.

7. Rat Ferritin Calibrator
   Add 1.0 mL of distilled or de-ionized water to the lyophilized Rat Ferritin Calibrator and mix gently until dissolved. The calibrator is now at a concentration of 2.660 µg/mL **(the reconstituted calibrator should be aliquoted and frozen if future use is intended).** Rat Ferritin calibrators need to be prepared immediately prior to use according to the chart below. Mix well between each step. Avoid foaming.

Calibrator	Concentration (ng/mL)	Calibrator Volume added to 1X Diluent →	Volume of 1X Diluent
6	400	100 µL Rat Ferritin Calibrator	565 µL
5	200	300 µL Calibrator 6	300 µL
4	100	300 µL Calibrator 5	300 µL
3	50	300 µL Calibrator 4	300 µL
2	25	300 µL Calibrator 3	300 µL
1	12.5	300 µL Calibrator 2	300 µL
0	0		600 µL

8. Positive Control
   The concentration and recommended dilution are provided on the Control Certificate. Before use, briefly centrifuge the Positive Control to allow all of the liquid to collect in the bottom of the vial.

STORAGE AND STABILITY

1. Complete Kit
   The expiration date for the kit is stated on the outer label. The recommended storage temperature is 4°C. **Note: See long term storage recommendations below for the Rat Ferritin Calibrator and Positive Control.**

2. Diluent
   The 5X Diluent Concentrate is stable until the expiration date. The 1X working solution is stable for at least one week from the date of preparation. Both solutions should be stored at 4°C.

3. Wash Solution
   The 20X Wash Solution Concentrate is stable until the expiration date. The 1X working solution is stable for at least one week from the date of preparation. Both solutions should be stored at 4°C.

4. Enzyme-Antibody Conjugate
   Undiluted anti-Ferritin-HRP conjugate should be stored at 4°C in the dark and **diluted immediately prior to use**. The undiluted conjugate solution is stable until the expiration date.

5. TMB Substrate Solution
   The TMB Substrate Solution should be stored at 4°C in the dark and is stable until the expiration date. Protect from light.

6. Stop Solution
   The Stop Solution should be stored at 4°C and is stable until the expiration date.

7. Microtiter Plate
   Anti-rat ferritin coated wells are stable until the expiration date and should be stored at 4°C in the sealed foil pouch with a desiccant pack.

8. Rat Ferritin Calibrator
   The lyophilized Rat Ferritin Calibrator should be stored at 4°C or frozen until reconstituted. The reconstituted calibrator should be aliquoted out and stored frozen (avoid multiple freeze-thaw cycles). The working calibrator solutions should be prepared immediately prior to use.

9. Positive Control
   For storage longer than 7 days keep frozen until the expiration date. Storage less than 7 days can be at 4°C. Avoid multiple freeze/thaw cycles.

INDICATIONS OF INSTABILITY

If the test is performing correctly, the results observed with the calibrator solutions should be within 20% of the expected values.

SPECIMEN COLLECTION AND HANDLING

Blood should be collected by venipuncture and the serum separated from the cells after clot formation by centrifugation. For plasma samples, blood should be collected into a container with an anticoagulant and then centrifuged. Care should be taken to minimize hemolysis, excessive hemolysis can impact your results. Assay immediately or aliquot and store samples at -20°C. Avoid repeated freezing/thawing.

For any sample that might contain pathogens, care must be taken to prevent contact with open wounds.

ASSAY PROTOCOL

Dilution of Samples

Due to the high sensitive nature of the assay each sample should be diluted before use for a normal assay. A 1:40 dilution is appropriate for most serum/plasma samples. For absolute quantification of samples that yield results outside the range of the calibration curve, a lesser or greater dilution might be required. **If unsure of sample level, a serial dilution with one or two representative samples before running the entire plate is highly recommended.**

To prepare a 1:40 dilution of sample, transfer 10 µL of sample to 390 µL of 1X Diluent. This gives you a 1:40 dilution. Mix thoroughly.

Procedure

1. **Bring all reagents to RT before use.**

   The Calibrators and the test sample(s) should be loaded into the ELISA wells as quickly as possible to avoid a shift in OD readings. Using a multichannel pipette would reduce this occurrence.

2. Pipette 100 µL of
   Calibrator 0 (0.0 ng/mL) in duplicate
   Calibrator 1 (12.5 ng/mL) in duplicate
   Calibrator 2 (25 ng/mL) in duplicate
   Calibrator 3 (50 ng/mL) in duplicate
   Calibrator 4 (100 ng/mL) in duplicate
   Calibrator 5 (200 ng/mL) in duplicate
   Calibrator 6 (400 ng/mL) in duplicate

3. Pipette 100 µL of diluted Positive Control (in duplicate) into pre designated wells.

4. Pipette 100 µL of diluted sample (in duplicate) into pre designated wells.
5. Incubate the Microtiter Plate at 22°C (RT) for sixty (60 ± 2) minutes. Keep plate covered and level during incubation.
6. Following incubation, aspirate the contents of the wells.
7. Completely fill each well with appropriately diluted Wash Solution and aspirate. Repeat three times, for a total of four washes. If washing manually; completely fill wells with wash buffer, invert the plate and pour/shake out the contents in a waste container. Follow this by sharply striking the wells on absorbent paper to remove residual buffer. Repeat 3 times for a total of 4 washes.
8. Pipette 100 µL of appropriately diluted Enzyme-Antibody Conjugate to each well. Incubate at 22°C (RT) for ten (10 ± 2) minutes. Keep plate covered in the dark and level during incubation.
9. Wash and blot the wells as described in Steps 6 and 7.
10. Pipette 100 µL of TMB Substrate Solution into each well.
11. Incubate in the dark at RT for precisely ten (10) minutes.
12. After ten (10) minutes, add 100 µL of Stop Solution to each well.
13. Determine the absorbance at 450 nm of the contents of each well within 30 minutes. Calibrate the plate reader to manufacturer's specifications.

The absorbance of the final reaction mixture can be measured up to 2 hours after the addition of the Stop Solution. However, good laboratory practice dictates that the measurement be made as soon as possible.

RESULTS

1. Subtract the average background value from the test values for each sample.
2. Using the results observed for the calibrators construct a calibration curve. The appropriate curve fit is that of a four-parameter logistics curve. A second order polynomial (quadratic) or other curve fits may also be used.
3. Interpolate test sample values from calibration curve. Correct for sample dilution factor to arrive at ferritin concentration in original sample.

QUALITY CONTROL

In accord with good laboratory practice, the assays for specific ferritin require meticulous quality control. Each laboratory should use routine quality control procedures to establish inter- and intra-assay precision and performance characteristics.

LIMITATION OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the information contained in the package insert instructions and with adherence to good laboratory practice.
2. Factors that might affect the performance of the assay include proper instrument function, cleanliness of glassware, quality of distilled or de-ionized water, and accuracy of reagent and sample pipettings, washing technique, incubation time or temperature.

FOR RESEARCH USE ONLY

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