



Rat Ferritin ELISA

Cat. No.: RSCPRA011R

1. INTRODUCTION

Ferritin, molecular weight 450,000, is iron storage protein containing 24 units of 2 different subunits (H:MW=21,000, L:MW=20,700) at various ratios. It contains 4,500 iron atoms in a molecule. Generally, ferritin exist in spleen, liver, red marrow, small intestinal mucosa and muscle, and involve in iron metabolism of inner or outer cellular. Ferritin is a source of supply for metallic iron, such as hemoglobin and enzymes containitng iron, and has a role to store iron, which catalyzes the hydroxy-radical synthesize reaction, into core of protein molecule. Determination of ferritin in serum is useful for diagnosis of disorder in iron metabolism, hepatic desease and malignant tumor.

2. CHARACTERISTICS

1. Since this includes an exclusive reagent for quantitative determination in rats, specific and precise data can be obtained.
2. Because of employing the EIA method. no special facility is necessary

3. COMPOSITION OF THE REAGENT KIT

1. ELISA plate (Anti-rat ferittin antibody -coated solid phase plate) 1 plate
2. Standard rat albumin (200 ng/ml) for 2 ml (lyophilized) 1 vial
3. Concentrated sample diluent buffer (5-fold concentrated, for 200 ml use) 1 vial
4. Enzyme - labeled antigen (Peroxidase-conjugated rat albumin) for 6ml use (lyophilized) 1 vial
5. Chromogen solution containing 13.2 mg of 3,3' ,5,5'-Tetramethylbenzidine in 1 ml N,N-dimethylformamide) 1 vial
6. Substrate solution (Containing 4.0 mg of hydrogen peroxide) 20 ml 1 vial
7. Concentrated washing solution (10-fold concentrated PBS-Tween 20, for 400 ml use) 1 vial
8. Stopping solution (1 mol/l sulfuric acid) 1 vial

4. REAGENT PREPARATION

Reagent	Method for preparation	Reagent prepared	Method and terms for valid storage
1. ELISA plate	Wait until the plate return to room temperature. Add 300 μ l of wash buffer into each wells just before use, and leave them for 10 minutes.	Anti-rat ferritin antibody-coated plate.	Prepare a necessary strips number, before use.
2 Standard rat ferritin	Accurately add 2.0 ml of purified water*, and mix in thoroughly for complete dissolution. Be careful not to bubble.	Standard rat ferritin (200 ng/ml)	Stable in refrigerator (2-10°C) for one week.
3. Sample diluent buffer	Add the whole volume of 40 ml into 160 ml of purified water, and mix it thoroughly.	Sample diluent	Stable in refrigerator (2-10°C).
4. Enzyme-labeled antibody	Accurately add 12 ml of purified water to vial, and mix thoroughly.	Enzyme-labeled antibody solution	Stable in refrigerator (2-10°C) for one week.
5. Chromogen solution 6. Substrate reagent	Collect 10.0 ml of the substrate solution. Add 100 μ l of chromogenic solution to it.	Chromogenic substrate solution	Freshly prepare it, just before use.
7. Wash buffer concentrate	Add the whole volume of 40 ml into 360 ml of purified water, and mix thoroughly it.	Wash buffer (PBS-0.05 v/v% Tween 20)	Stable at room temperature for one week.
8. Stop solution	Use it as it is	-----	Stable at room temperature.

5. NECESSARY INSTRUMENT AND APPARATURES

1. Micro pipette and tips (50 μ l, 100 –1000 μ l)
2. Mass pipette (1 ml, 10 ml)
3. Mass cylinder (500 ml)
4. Cleaning instrument for 96 wells microtiter plate
(In the case of manual operation: Continuous distributor and aspirator, etc.)
5. Multi-channel pipette
6. Microtiter plate reader (With measuring wave length of 450 nm)

6. ASSAY PROCEDURE

6.1. Preparation of standard rat ferritin

Accurately add 2.0 ml of distilled or deionized water into the vial containing the standard rat ferritin, providing the concentration of 200 ng/ml. Dilute the original solution in a series so as to prepare varying dilutions of 100, 50, 25, 12.5, 6.25, 3.13 ng/ml. For 0 ng/ml, use the sample diluent.

6.2. Preparation of samples

Blood sample

Use serum. Do not use the plasma collected using EDTA as an anticoagulant. It will remain stable for 3 months when kept stored below -20°C. As the influences of serum components are deemed likely, dilute the sample more than 20-fold. When the sample concentration is too high and come out of the measurement range, dilute the sample with the sample diluent.

Add 950 µl of the sample diluent into 50 µl of serum (20-fold dilution).

6.3. Assay protocol

It is recommended to conduct all the measurements in duplicity or in a higher multiplicity.

1. Add 300 µl of the wash buffer to each well of the ELISA plate. Incubate for 10 minutes at room temperature. (There is no adverse effect, even when it is left standing for up to 30 minutes.)
2. Aspirate the solution.
3. Add 100 µl of the standard rat ferritin or the unknown sample to each well, and incubate for 2 hours at room temperature.
4. Aspirate the solution, and wash well 3 times with wash buffer (300 µL/well/wash). Aspirate and tap firmly after each wash to remove residual buffer.
5. Add 100 µl of the enzyme-labeled antibody to each well, and incubate for one hour at room temperature.
6. Wash step as in step 4.
7. Add 100 µl of the chromogenic substrate solution to each well and incubate at room temperature for 15 minutes.
8. Add 50 µl of the stop solution to each well.
9. Measure the absorbance at 450 nm with a microfilter plate reader.

7. CALCULATION OF RESULTS

1. Average the duplicate reading for each standard, sample.
2. Plot the value of absorbance (Y-axis) against the concentration of the standard solution (X-axis), thus prepare the standard curve.
3. Apply the values of the absorbance of the sample into the standard curve, so as to read the rat ferritin concentration in the sample, and multiply this concentration by the dilution multiple.

8. SAFETY WARNINGS AND PRECAUTIONS

1. Strictly observe the term and the method for storage for each reagent
2. All reagents should be brought to room temperature before use.
3. Use reagent after confirming that each of them is completely dissolved.
4. Take care to not inflict damage to any well when aspirating the solution in each well.
5. For measurement of many samples, take care that the reaction time of each sample is at a fixed time as designated.
6. Prepare the standard curve freshly for every measurement.
7. Prepare the chromogenic substrate solution with a clean instrument before use. (The substrate reagent may be developed due to contamination of the instrument.)
8. White powder may sometimes be found on the wells. This is due to the dried block solution, but will not have an effect on measurement.
9. As the stop solution is 1 mol/l sulfuric acid, take care to handle.

9. ADDITIONAL INFORMATION

9.1. Range of measurement

Within the range of 3.13–200 ng/ml, rat ferritin can be measured with this system.

9.2. Intra - assay precision

standard		
Rat ferritin (ng/mL)	Mean value of absorbance	(%) C.V.
0 (N=8)	0.058	10.3
3.13 (N=8)	0.266	2.3
6.25 (N=8)	0.347	4.0
12.5 (N=8)	0.517	6.0
25 (N=8)	0.761	3.9
50 (N=8)	1.040	2.5
100 (N=8)	1.339	2.3
200 (N=8)	1.627	2.7
sample		
Serum	Mean value of absorbance	(%) C.V.
A (N=8)	0.641	3.3
B (N=8)	1.101	3.5
Serum		
A (N=8)	18.22	6.0
B (N=8)	57.34	8.5

(%) C.V.= coefficient of variation

9.3. Inter-assay precision

standard		
Rat ferritin (ng/mL)	Mean value of absorbance	(%) C.V.
0 (N=8)	0.064	7.8
3.13 (N=8)	0.222	11.7
6.25 (N=8)	0.337	13.1
12.5 (N=8)	0.501	10.6
25 (N=8)	0.726	7.4
50 (N=8)	0.998	8.2
100 (N=8)	1.287	7.3
200 (N=8)	1.570	5.9
sample		
Serum	Mean value of absorbance	(%) C.V.
A (N=8)	0.634	9.6
B (N=8)	1.079	7.2
Serum		
A (N=8)	19.26	13.5
B (N=8)	61.57	10.5

(%) C.V.= coefficient of variation

1. The "A" represents the sample of SD rats (male at age of 7 weeks) serum, with dilution into 20-fold volume.
2. The "B" represents the sample prepared by addition of the standard rat ferritin to the 20-fold diluted SD rats (male at age of 7 weeks) serum.

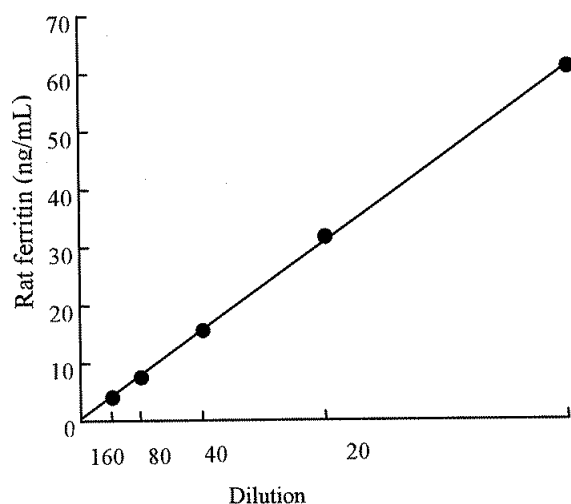
9.4. Test of recovery after addition

The results of measurement with addition of the standard rat ferritin to SD rats (male at age of 7 weeks) serum, diluted more than 20-fold.

Serum	Amount of addition (ng/mL)	Value of actual measurement (ng/mL)	Theoretical Value (ng/mL)	Recovery ratio (%)
1	0	20.83	-	-
	25	44.64	45.83	97.4
	50	69.68	70.83	98.4
	100	117.13	120.83	96.9
2	0	8.25	-	-
	1.56	10.23	9.81	104.3
	6.25	15.17	14.50	104.7
	25.0	36.30	33.25	109.2
3	0.0	3.75	-	-
	1.6	4.68	5.31	88.2
	3.13	7.38	6.88	107.3
	12.5	18.08	16.25	111.3

9.5. Dilution test

Within the range of with dilution of SD rats (male at age of 7 weeks) serum into 10-160-fold volume by the sample diluent.



10. STORAGE AND EXPIRY

Stability is assured until the demonstrated expiration date (one year after manufacture), following storage in the dark and cool place (2-10°C).

11. PACKAGE

96 units for test.

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