Rat Transferrin ELISA

Cat. No.: RSCPRG091R

1. INTRODUCTION

Transferrin is a protein which transports iron-complexed of 1-fraction of blood, and which have molecular weights of 70,000-90,000. Only iron molecules-transferrin complex can be utilized by erythroblasts and reticulocytes for synthesis of hemoglobin in the body. Furthermore, transferrin sensitively reflects the level of iron stored in the body, and thus serves as an important index for iron metabolism and hematopoiesis. In addition, transferrin is known as an important substance for cell proliferation and bio-immunological defense of the human body, and thus reflects various pathological conditions.

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.

3. COMPOSITION OF THE REAGENT KIT

1. ELISA plate (96 well coated with anti-rat transferring antibody) x 1 plate
2. Standard rat transferrin (40 ng/mL) for 2 mL (lyophilized) x 1 vial
3. Sample diluent concentrate (10-fold concentrated, for 400 mL use) 40 mL x 1 vial
4. Enzyme-labeled antibody (Peroxidase-conjugated anti-rat transferrin antibody, for 12 mL use)(lyophilized) × 1
5. Chromogen solution (Containing 13.2 mg of 3,3',5,5'-Tetramethylbenzidine in 1 mL N,N-dimethylformamide) 500 µL x 1 vial
6. Substrate solution (Containing 4.0 mg of hydrogen peroxide) 20 mL 1 vial
7. Wash buffer concentrate (10-fold concentrated PBS-Tween 20, for 400 mL use) 40 mL x 1 vial
8. Stop solution (1 mol/L sulfuric acid) 15 mL x 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 µL of wash buffer into each wells just before use, and leave them for 10 minutes.	Anti-rat transferrin antibody-coated plate.	Prepare a necessary strips number, before use.
2 Standard rat transferrin	Accurately add 2.0 mL of purified water*, and mix in thoroughly for complete dissolution. Be careful not to bubble.	Standard rat transferrin (20 ng/mL)	Stable in refrigerator (2-10°C) for one week.
3. Sample diluent buffer	Add the whole volume of 40 mL into 360 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 3.0 mL of the substrate solution. Add 30 µL 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.

NOTE: *: Distilled or deionized water

All reagents should be allowed to equilibrate to room temperature before use.

The unnecessary strips should be closed up in the foil pouch and stored at 2-10°C protected from light.

Do not store chromogenic substrate solution after mixing (5) with (6)

5. NECESSARY INSTRUMENT AND APPARATURES

1. Micro pipette and tips (50 µL, 100 – 1000 µL)
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 aspirator, etc.)
5. Multi-channel pipette
6. Microtiter plate reader (With measuring wave length of 450 nm)

6. OPERATION METHOD FOR MEASUREMENT

6.1 Preparation of standard rat transferrin solution

Accurately add 2.0 mL of distilled or deionized water into the vial containingt the standard rat transferrin, providing the concentration of 40 ng/mL. Dilute the original solution in a series so as to prepare varying dilutions of 20, 10, 5, 2.5, 1.25, 0.63 ng/mL. For 0 ng/mL, use the sample diluent.

6.2 Preparation of samples

Fece sample

Suspend the fresh feces with the sample diluent and apply it centrifugal. Use the supernatant as the sample. Store the samples below -20°C prior to analysis. As the rat transferrin expected to be in the order of µg per gram of feces, dilute the samples depending on the expected concentration.

Urine sample

Centrifuge fresh urine or pooled urine (for example, at 2,500 rpm. at 4°C for 15 minutes), and use the supernatant as the sample. Store the samples below -20°C prior to analysis. As the rat transferrin concentration is expected to be in the order of µg per mL of urine, dilute the samples depending on the expected concentration.

Blood sample

Presence of anticoagulants or similar do not exert any influence upon the values of measurement. Separate both serum and plasma from blood cells as quickly as possible after the blood sample collection. Specimens should be stored below –20°C in case of storage for long period. When the blood samples are collected with heparin, fibrin may precipitate during storage. At the time of thawing, watery components may gather into the lower layer. Therefore, thoroughly mix the sample

with stirring before measurement. As the rat transferrin concentration is expected to be in the order of µg per mL of urine, dilute the samples depending on the expected concentration.

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). Aspitrate and top 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 it.

9. PERFORMANCE OF THE SYSTEM

9.1 Range of measurement

Within the range of 0.625–40 ng/mL, rat transferrin can be measured with this system.

9. 2. Intra - assay precision

standard Rat transferrin (ng/mL)		Mean valueof absorbance	(%) C.V.
0	(N=8)	0.048	2.1
0.63	(N=8)	0.136	2.9
1.25	(N=8)	0.219	1.4
2.5	(N=8)	0.316	3.2
5	(N=8)	0.512	1.8
10	(N=8)	0.804	1.6
20	(N=8)	1.133	1.1
40	(N=8)	1.332	1.2
sample Fese		Mean valueof absorbance	(%) C.V.
A	(N=8)	0.169	1.8
B	(N=8)	0.469	3.2
Fese			
A	(N=8)	0.909	3.2
B	(N=8)	4.878	4.4

(%) C.V.= coefficient of vatiation

9.3 Inter - assay precision

standard Rat transferrin (ng/mL)		Mean valueof absorbance	(%) C.V.
0	(N=8)	0.060	11.7
0.63	(N=8)	0.117	8.5
1.25	(N=8)	0.172	10.5
2.5	(N=8)	0.268	9.0
5	(N=8)	0.432	8.1
10	(N=8)	0.681	7.8
20	(N=8)	0.980	6.3
40	(N=8)	1.232	5.5
sample Fese		Mean valueof absorbance	(%) C.V.
A	(N=8)	0.173	8.7
B	(N=8)	0.450	7.3
Fese			
A	(N=8)	1.184	16.9
B	(N=8)	5.234	5.8

(%) C.V.= coefficient of vatiation

1. The“A”represents the sample prepared from one gram of SD rats (male at age of 7 weeks) feces suspended with 20mL of sample diluent, and with further dilution into 100-fold volume.
2. The“B”represents the sample prepared from one gram of of SD rats (male at age of 7 weeks) feces suspended with 20 mL of the sample diluent, and with further dilution into 100-fold volume and addition of standard rat transferrin.

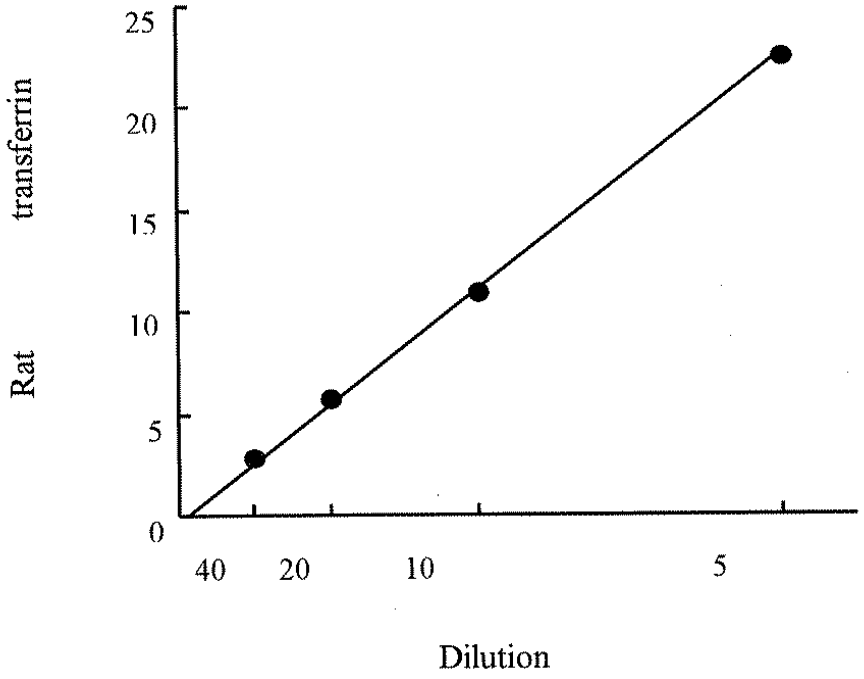
9.4 Test of recovery after addition

The results of measurement with addition of the standard rat transferrin to the feces of SD rats (male at age of 7 weeks).

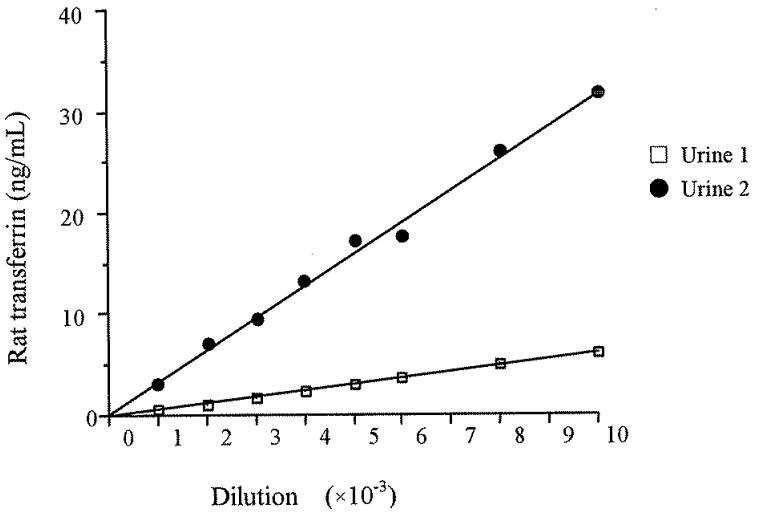
Feces	Amount of addition (ng/mL)	Value of actual measurement (ng/mL)	Theoretical Value (ng/mL)	Recovery ratio (%)
	0	2.17	-	-
	0.63	2.81	2.80	100.0
1	1.25	3.46	3.42	101.0
	2.5	4.65	4.67	99.6

9.5 Dilution test

The results of measurement with 0.2 g of SD rats (male at age of 7 weeks) feces suspended with 4 mL of sample diluent and with dilution into 5, 10, 20 and 40-fold volumes.



The results of measurement with dilution of SD rats (male at age of 7 weeks) urine into 100-fold volume by sample diluent, and with further dilutions.



10. METHOD FOR STORAGE AND TERMS OF VALIDITY

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.

Gentaur Molecular Products
Voortstraat 49
1910 Kampenhout, Belgium
http://www.gentaur-worldwide.com