

Kit for measurement of Rat Hemoglobin according to EIA method
PRC031

Pana-test R a t H e m o g l o b i n

1. Introduction

Examination of occult blood in urine is used as a screening test for hemorrhagic lesion of gastrointestinal tract and renal disturbance. However, it is said that there is problem in confidence of data, because chemical method usually applied shows frequent quasi-positive or quasi-negative due to presence of various components in urine. This reagent can detect occult blood specifically and highly sensitivity by the EIA method with specific antibody to rat hemoglobin.

2. Characteristics

1. Since this includes an exclusive reagent for quantitative determination in rats, specific and precise data can be obtained.
2. No special facility is necessary, due to EIA method.

3. Composition of the reagent kit

1. ELISA plate (Anti-rat hemoglobin antibody-coated solid phase plate) 96wells \times 1
2. Standard rat hemoglobin (160ng/mL) for 2mL (lyophilized) \times 1
3. Powder of sample diluent buffer (Block Ace) 4g \times 3
4. 10% SDS of sample diluent buffer 8mL \times 1
5. Enzyme - labeled antibody (Peroxidase-conjugated anti-rat hemoglobin antibody) for 12mL use (lyophilized) \times 1
6. Chromogenic tablet (Containing 10mg of *o*-phenylenediamine in a tablet) for 8mL/tablet \times 2
7. Substrate solution (Containing 4.0mg of hydrogen peroxide) 20mL \times 1
8. Concentrated washing solution (10-fold concentrated PBS-Tween 20, for 400mL use) 40mL \times 1
9. Stopping solution (2N sulfuric acid) 15mL \times 1

4. Method for preparation of test reagents

Test reagent	Method for preparation	Reagent prepared	Method and terms for valid storage
① ELISA plate	Remove from aluminum package and wait until plate reaches room temperature. Put 300 μ L of washing solution into each of 96wells just before use, and leave them standing for 10 minutes.	Plate for changing into solid phase	Prepare wells in a necessary number, freshly before use.
② Standard rat hemoglobin	Accurately add 2.0mL of purified water*. Thoroughly mix it for complete dissolution, with care not to form any bubbles.	Standard rat hemoglobin 160ng/mL	Solution is stable for one week when stored in the dark and cool place (2-10°C).
③ Powder of sample diluent buffer ④ 10%SDS of sample diluent buffer	Add a bag of Block Ace(4g) into 98mL of purified water*. Add 2mL of dissolved 10% SDS after returning to room temperature into it, and mix it thoroughly.	Sample diluent buffer	Stable in the dark and cool place (2-10°C).
⑤ Enzyme-labeled antibody	Accurately add 12mL of purified water* to vial, and mix it thoroughly.	Enzyme-labeled antibody solution	Antibody solution is stable for one week in the dark and cool place (2-10°C).
⑥ Chromogenic tablet ⑦ Substrate solution	Collect 8mL of the substrate solution. Add it to one chromogenic tablet. Thoroughly mix for complete dissolution.	Chromogenic substrate solution	Freshly prepare it, just before use.
⑧ Concentrated washing solution	Add the whole volume of 40mL into 360mL of purified water*, and thoroughly mix it.	Washing solution (PBS-0.05% Tween 20)	Washing solution is stable for one month at room temperature.
⑨ Stopping solution	Use it in its intact form.		Stable at room temperature.

Important notes

* : Distilled or deionized water

Make sure that all the test reagents are used after returning to room temperature.

(10%SDS is precipitated in low temperature, make sure it is used after returning to room temperature.)

Wells not used out of those of the ELISA plate must be kept tightly-sealed in the aluminum package in which the plate was kept, and must be kept in the dark and cool place.

The chromogenic substrate should not be stored for prolonged period after mixing ⑤ with ⑥.

5. Necessary instrument and apparatuses

1. Micro pipette and tips (50 μ L, 100 μ L, 100-1000 μ L)
2. Mass pipette (2mL, 10mL)
3. Mass cylinder (100mL, 500mL)
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 492nm)

6. Operation method for measurement

Preparation of standard rat hemoglobin solution

Accurately add 2.0mL of distilled or deionized water into the vial containing the ② standard rat hemoglobin providing the concentration of 160ng/mL. Dilute the original solution multiply in a series so as to prepare varying dilutions of 80ng/mL, 40ng/mL, 20ng/mL, 10ng/mL, 5ng/mL and 0ng/mL. For 0ng/mL, use the ③ ④ sample diluent buffer.

Preparation of test sample

Use urine. Store the test sample below -20°C. Dilute the test sample more than 10-fold. For the test sample in high concentration which are assumed to be so high as to be out of the measurable range, dilute the test sample with ③ ④ sample diluent buffer.

Procedures for measurement operation

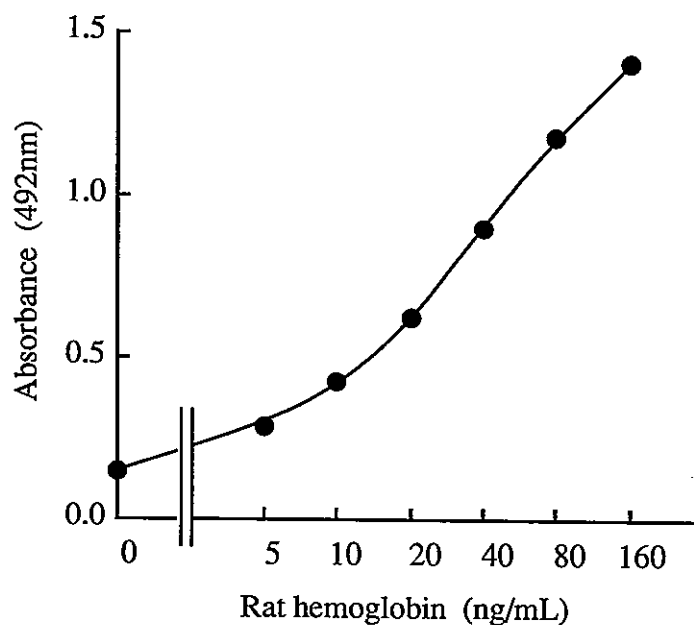
It is recommended to conduct all the measurements in duplicity or in a higher multiplicity.

- 1) Take the ① ELISA plate out of the aluminum package after reaching room temperature, and distribute 300 μ L each of the ⑧ washing solution into each well to be used. Leave them standing for 10 minutes at room temperature. (There is no adverse effect, even when it is left standing for up to 30 minutes.)
- 2) Remove the liquid in the well by suction with an aspirator.
- 3) Add 100 μ L each of the ② standard rat hemoglobin or the test sample (after necessary dilutions) into each well, and leave them standing for 2 hours at room temperature.

- 4) Remove the liquid in the well by suction with an aspirator, and distribute 300 μ L each of the ⑧ washing solution into each well. Then, remove the ⑧ washing solution from each well.
- 5) Repeat the same step as in 4) twice, for further washing.
- 6) Add 100 μ L each of the ⑤ enzyme-labeled antibody into each well, and leave them standing at room temperature for one hour.
- 7) Remove the liquid in the well by suction with an aspirator, and distribute 300 μ L each of the ⑧ washing solution into each well. Then, remove the washing solution from each well.
- 8) Repeat the same step as in 7) twice, for further washing.
- 9) Add 100 μ L each of the ⑥ and ⑦ chromogenic substrate solution, prepared as above, into each well in the designated order and at a fixed intervals, reacting at room temperature for 15 minutes.
- 10) Add 50 μ L each of the ⑨ stopping solution into each well in the same order and at the same interval as the addition of the chromogenic substrate solution, so as to stop the enzyme reaction.
- 11) Measure the absorbance at 492 nm with a microtiter plate reader.

7. Method for calculation of rat hemoglobin concentration

- 1) Calculate the mean value of each absorbance obtained by the duplicate measurements.
- 2) Plot the concentration of the standard solution on the X-axis and the value of the absorbance on the Y-axis, to thus prepare the standard curve.
- 3) Apply the values of the absorbance of the test sample into the standard curve, so as to read the rat hemoglobin concentration in the test sample and multiply this concentration by the dilution multiple.



8. Precautions for the measurement

- 1) Strictly observe the term and the method of storage for each test reagent.
- 2) Make sure to return the prepared test reagents to room temperature before actual use.
- 3) Use each test reagent after confirming that each of them is completely dissolved.
- 4) Take care to not inflict damage to any well when removing the reaction solution from each well by suction.
- 5) For measurement of many test samples, take care that the reaction time of each test sample is at a fixed time as designated.
- 6) Prepare the standard curve freshly for each measurement.
- 7) Thoroughly clean the instrument for preparation of Chromogenic substrate solution before actual use.
(Color development may take place due to contamination of the instrument.)
- 8) White powder may sometimes be found, adhered to the wells of the ELISA plate. This is due to the dried blocking solution, but will not give any adverse effect upon the measurement.
- 9) As the ⑨ stopping solution is 2N sulfuric acid, take care when handling it.

9. Performance of the system

Range of measurement

Within the range of 5 - 160ng/mL, rat hemoglobin can be measured with this system.

Intra - assay precision

Standard			
Rat hemoglobin (ng/mL)		Mean value of absorbance	C.V.(%)
0	(N=8)	0.143	4.9
5	(N=8)	0.276	2.5
10	(N=8)	0.381	1.8
20	(N=8)	0.574	4.0
40	(N=8)	0.836	3.1
80	(N=8)	1.100	2.5
160	(N=8)	1.328	2.5

C.V. = Coefficient of variation

Test sample

urine		Mean value of absorbance	C.V.(%)
A	(N=8)	0.330	1.5
B	(N=8)	0.833	1.7
urine		Mean value of concentration(ng/mL)	C.V.(%)
A	(N=8)	7.64	3.2
B	(N=8)	40.11	3.5

The urine A represents the test sample of serum of SD rats (male at age of 7 weeks), with dilution into 20-fold volume.

The urine B represents the test sample prepared by addition of the standard rat hemoglobin to the 20-fold diluted urine of SD rats (male at age of 7 weeks).

Inter - assay precision

Standard			
Rat hemoglobin (ng/mL)		Mean value of absorbance	C.V.(%)
0	(N=8)	0.151	4.0
5	(N=8)	0.286	4.2
10	(N=8)	0.426	4.9
20	(N=8)	0.628	4.5
40	(N=8)	0.899	5.9
80	(N=8)	1.175	5.6
160	(N=8)	1.408	5.7

C.V. = Coefficient of variation

Test sample			
urine		Mean value of absorbance	C.V.(%)
A	(N=8)	0.369	4.6
B	(N=8)	0.893	4.7

urine		Mean value of concentration(ng/mL)	C.V.(%)
A	(N=8)	7.88	8.7
B	(N=8)	39.71	4.0

The urine A represents the sample of serum of SD rats (male at age of 7 weeks), with dilution into 20-fold volume.

The urine B represents the test sample prepared by addition of the standard rat hemoglobin to the 20-fold diluted urine of SD rats (male at age of 7 weeks).

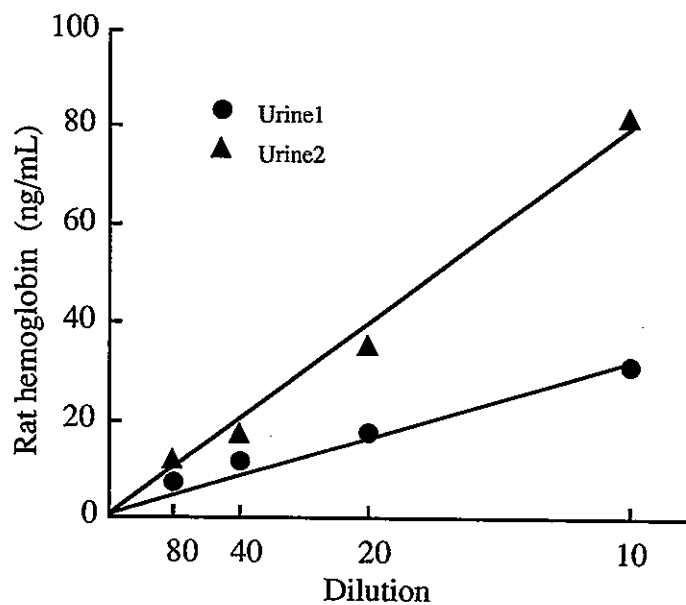
Test of recovery after addition

The results of measurement with addition of the standard rat hemoglobin to the urine of SD rats (male at age of 7 weeks), diluted more than 10-fold.

urine	Amount of addition (ng/mL)	Value of actual measurement(ng/mL)	Theoretical value (ng/mL)	Recovery ratio (%)
1	0	12.2	-	-
	10	21.0	22.2	94.6
	20	30.8	32.2	95.7
	40	55.1	52.2	105.6
2	0	8.5	-	-
	10	17.9	18.5	96.8
	20	29.2	28.5	102.5
	40	48.9	48.5	100.8
3	0	12.5	-	-
	10	23.0	22.5	102.2
	20	32.6	32.5	100.3
	40	56.7	52.5	108.0

Dilution test

Within the range of with dilution of urine of SD rats (male at age of 7 weeks) into 10 - 80-fold volume by the sample diluent buffer, the straight line of dilution can be measured.



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.