KAMIYA BIOMEDICAL COMPANY

Rat Hemoglobin ELISA

For the quantitative determination of hemoglobin in rat urine

Cat. No. KT-449

For research use only, not for use in diagnostic procedures.

PRODUCT INFORMATION

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PRODUCT
The **K-*ASSAY*®** Rat Hemoglobin ELISA is for the quantitative determination of hemoglobin in rat urine.

PRINCIPLE
Examination of occult blood in urine is used as a test for hemorrhagic lesion of gastrointestinal tract and renal disturbance. However, it is said that there is a problem in the confidence of data, because the chemical method usually applied shows frequent quasi-positive or quasi-negative due to the presence of various components in urine. This reagent can detect occult blood specifically and is highly sensitive using the ELISA method with a specific antibody to rat hemoglobin. Since this kit includes an exclusive reagent for quantitative determination in rats, specific and precise data can be obtained. No special facility is necessary due to the ELISA method.

COMPONENTS
- Microtiter Plate: Anti-rat hemoglobin antibody-coated solid phase plate, 96-wells
- Rat Hemoglobin Calibrator: 160 ng/mL for 2 mL (lyophilized)
- Sample Diluent Buffer Powder (Block Ace), 3 x 4 g
- Sample Diluent Buffer 10% SDS, 8 mL
- Enzyme-labeled antibody: Peroxidase-conjuagted anti-rat hemoglobin antibody, 12 mL (lyophilized)
- Chromogen Solution: 13.2 mg 3,3',5,5'-tetramethylbenzidine in 1 mL N,N-dimethylformamide, 500 µL
- Substrate Solution: 4.0 mg hydrogen peroxide, 20 mL
- Concentrated Washing Solution: 10-fold concentrated PBS, Tween 20, 40 mL (for 400 mL use)
- Stop Solution: 1M Sulfuric Acid, 15 mL

Materials or Equipment required but not provided
- Micropipette and tips (50 µL, 100 µL, 100-1,000 µL)
- Mass pipette (2 mL, 10 mL)
- Mass cylinder (100 mL, 500 mL)
- Cleaning instrument for 96 wells microtiter plate (In case of manual operation: continuous distributor aspirator, etc.)
- Microtiter plate reader (450 nm)
- Multi-channel pipette

PREPARATION OF REAGENTS
A. Test Sample

1. Use urine. Store the test sample below -20℃.
2. Dilute the sample more than 10 fold. (For test samples with high concentrations which are assumed to be so high that they out of the measurable range, dilute the test sample with the sample diluent buffer.

B. Rat Hemoglobin Calibrator

1. Accurately add 2.0 mL of distilled or de-ionized water to the lyophilized calibrator to give a concentration of 160 ng/mL. Dilute the original solution in series to prepare varying dilutions of 80, 40, 20, 10, and 5 ng/mL. Use the sample diluent buffer as the 0 ng/mL calibrator.

Reagent	Method for preparation	Reagent prepared	Method and terms for valid storage
① ELISA plate	Wait until the plate returns to room temperature. Add 300 µL of wash buffer into each wells just before use, and leave them for 10 minutes.	Anti-rat hemoglobin antibody-coated plate	Prepare a necessary strips number,before use.
② Standard rat hemoglobin	Accrately add 2.0 mL of purified water* , and mix it thoroughly for complete dissolution. Be careful not to bubble.	Standard rat hemoglobin (160 ng/mL)	Stable in refrigerator (2–10℃) for one week
③sample diluent buffer	Add bag block ace (4g) into 98 mL of purified water. Add 2mL of dissolved 10% SDS after returning to room temperature into it, and mix thorougly it.	Sample diluent	Stable in refrigerator (2–10℃)
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℃) 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 thorougly 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 shoule be closed up in the foil pouch and stored at 2–10℃ protected from light.
Do not store chromogenic substrate solution after mixing ⑤ with ⑥

PROCEDURE

1. It is recommended to conduct all measurements in duplicity or in higher multiplicity.
2. Take the ELISA plate out of the aluminum package after reaching room temperature, and distribute 300 µL of washing solution into each well to be used. Let stand for 10 mins at room temperature. (There is no adverse affect even if left standing for up to 30 mins.)
3. Remove the liquid in the well by suction with an aspirator.
4. Add 100 µL of rat hemoglobin calibrator or the test sample (after necessary dilutions) into each well. Let stand for 2 hours at room temperature.
5. Remove the liquid in the well by suction with an aspirator, and distribute 300 µL of washing solution into each well. Then, remove the washing solution from each well.
6. Repeat step 5 twice for further washing.
7. Add 100 µL of enzyme-labeled antibody into each well. Let stand at room temperature for one hour.
8. Remove the liquid in the well by suction with an aspirator, and distribute 300 µL of washing solution into each well. Then, remove the washing solution from each well.
9. Repeat step 8 twice for further washing.
10. Add 100 µL of chromogenic substrate solution into each well in the designated order and at fixed intervals, reacting at room temperature for 15 mins.
11. Add 50 µL of 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.
12. Measure the absorbance at 450 nm with a microtiter plate reader.

CALCULATION OF RESULTS

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

PERFORMANCE CHARACTERISTICS

1. 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
40	(N=8)	0.836	3.1
80	(N=8)	1.100	2.5
160	(N=8)	1.328	2.5
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 absorbance	(%) C.V.
A	(N=8)	7.64	3.2
B	(N=8)	40.11	3.5

(%) C.V.= Coefficient opf variation

Urine A represents the test sample of serum of SD rats (male, 7 weeks of age), with dilution into 20 fold volume.
Urine B represents the test sample prepared by addition of the rat hemoglobin calibrator to 20 fold diluted urine of SD rats (male, 7 weeks of age).

2. 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
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 absorbance	(%) C.V.
A	(N=8)	7.88	8.7
B	(N=8)	39.71	4.0

(%) C.V.= Coefficient opf variation

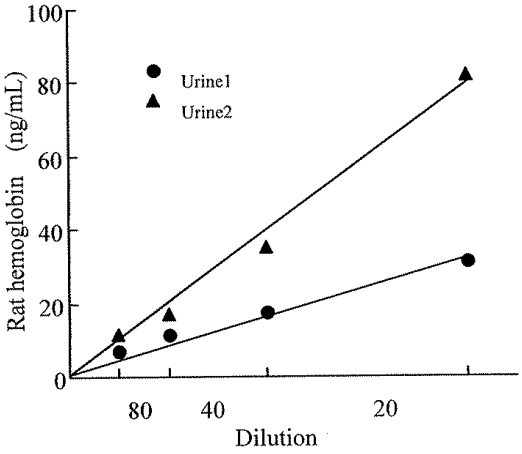
Urine A represents the test sample of serum of SD rats (male, 7 weeks of age), with dilution into 20 fold volume.
Urine B represents the test sample prepared by addition of the rat hemoglobin calibrator to 20 fold diluted urine of SD rats (male, 7 weeks of age).

3. Test of recovery after addition

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

The results of measurement with addition of rat hemoglobin calibrator to the urine of SD rats (male, 7 weeks of age), diluted more than 1 fold.

4. Dilution Test



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

TECHNICAL HINTS

- Strictly observe the term and method of storage for each test reagent.
- Make sure to return the prepared test reagents to room temperature before actual use.
- Use each test reagent after confirming that each of them is completely dissolved.
- Take care to not inflict damage to any well when removing the reaction solution from each well by suction.
- For measurement of many test samples, take care that the reaction time of each test sample is at a fixed time as designated.
- Prepare the calibration curve freshly from each measurement.
- Thoroughly clean the instrument for preparation of Chromogenic substrate solution before actual use. (Color development may take place due to contamination of the instrument.)
- 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 effects upon the measurement.
- As the stopping solution is 1M sulfuric acid, be cautious when using it.

STORAGE

Store at 4℃ in a dark and cool place. The kit is stable as supplied until the expiration date.

FOR RESEARCH USE ONLY

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