Pana-test Rat β_2-Microglobulin

1. Introduction

β_2-Microglobulin occurs in blood, urine, and other body fluids. It has been reported that, in β_2-microglobulin concentration in human blood varies under pathological conditions, such as glomerular disturbances, autoimmune diseases, malignant tumors and hepatic disturbances, meanwhile, its concentration in urine is subject to changes of urethral disturbances and its plasma concentration. This ELISA kit enables a quantitative analysis of rat β_2-microglobulin, based on the enzyme immunoassay (EIA) method with a specific antibody to rat β_2-microglobulin.

2. Characteristics

- This kit includes an exclusive reagent for quantitative determination of rat β_2-microglobulin.
- No specific facility is necessary.

3. Components of the Kit

- ELISA plate (anti-rat β_2-microglobulin antibody-coated microplate) ..... 1 plate
- Standard rat β_2-microglobulin (20 ng/mL) for 2 mL (lyophilized) ..... 1 vial
- Sample diluent concentrate, 40 mL (5-fold concentrated, for 200 mL use) ..... 1 vial
- Enzyme-labeled antibody (peroxidase-conjugated anti- rat β_2-microglobulin antibody) for 12 mL (lyophilized) ..... 1 vial
- Chromogen solution (containing 13.2 mg 3,3',5,5'-tetramethylbenzidine in 0.5 mL of *N,N*-dimethylformamide) ..... 1 vial
- Substrate solution, 20 mL (containing 0.0083 w/v% hydrogen peroxide) ..... 1 vial
- Washing buffer concentrate, 40 mL (10-fold concentrated PBS-Tween 20, for 400 mL use) ..... 1 vial
- Stop solution, 15 mL (1 mol/L sulfuric acid) ..... 1 vial

4. Reagent Preparation

Component	Preparation	Reagent prepared	Storage condition and stability
① ELISA plate	Wait until the plate reaches to room temperature. Add 300 µL of wash buffer to each well just before use, and leave for 10 minutes.	Anti- rat β_2-microglobulin antibody-coated plate	Prepare a required number of strip only immediately before use
② Standard rat β_2-microglobulin	Add accurately 2.0 mL of purified water [1] to the vial, and mix it thoroughly for complete dissolution. Be careful not to be bubbled.	Standard rat β_2-microglobulin (20 ng/mL)	Stable in a refrigerator (2 to 10°C) for one week
③ Sample diluent concentrate	Add the entire volume of the concentrate (40 mL) into 160 mL of purified water, and mix it thoroughly.	Sample diluent	Stable in a refrigerator (2 to 10°C) for one week
④ Enzyme-labeled antibody	Add accurately 12 mL of purified water to the vial, and mix it thoroughly.	Enzyme-labeled antibody solution	Stable in refrigerator (2 to 10°C) for one week
⑤ Chromogen solution ⑥ Substrate solution	Add 100 µL of the chromogen solution into 10 mL of the substrate solution.	Chromogenic substrate solution	Freshly prepare, just before use
⑦ Wash buffer concentrate	Add the entire volume of the concentrate (40 mL) into 360 mL of purified water, and mix it thoroughly.	Wash buffer [2]	Stable at room temperature for one week
⑧ Stop solution	Use it as it is		Stable at room temperature

1) Distilled or deionized water
2) PBS containing 0.05 v/v% Tween 20

NOTE: *: Distilled or deionized water
All reagents should be allowed to equilibrate to room temperature before use.
Disused strips should be closed up in the foil pouch and stored at 2 to 10°C under dark.
Immediately use the chromogenic substrate solution after mixing ⑤ with ⑥.

5. Supplies Required

- Micropipettes and pipette tips (50 µL, 100 to 1,000 µL)
- Blowout pipettes (2 mL, 10 mL)
- Graduated cylinder (500 mL)
- Squirt bottle, manifold dispenser, or automated microplate washer
- Multi-channel pipette
- Microplate reader capable of measurement at or near 450 nm
- Distilled or deionized water

6. Assay Procedure

6.1 Preparation of Standard Rat β_2-Microglobulin Solutions

Reconstitute the standard rat β_2-microglobulin with accurately 2.0 mL of deionized or distilled water, producing 20 ng/mL standard. Swirl or mix gently and leave for a while to ensure complete reconstitution. Make serial dilutions of the 20 ng/ mL standard with the sample diluent, to prepare the standard solutions at 10, 5, 2.5, 1.25, 0.63 and 0.31 ng/mL.
Distribute the sample diluent to the 0 ng/mL standard.

6.2 Sample Dilution

Blood sample

Commonly use serum or plasma (containing heparin or EDTA as an anticoagulant) as a sample for measurement of β_2-microglobulin. These sample should be diluted more than 1,500-fold the influences of blood components. As the content of β_2-microglobulin in these blood sample is expected to be in the order of μg in one mL of the test sample, dilute it whenever deemed necessary.

ex.) Add 0.8 mL of the sample diluent to 20 μL of serum (41-fold dilution). Then add 0.8mL of the sample diluent into 20 μL of the 41-fold dilute sample (1,681-fold dilution). (As carry-over of the test sample may be possible, it is recommended to replace the chip for each dilution.)

Urine sample

Use optionally collected urine or 24 hours pooled urine. Adjust pH of the test sample to 6.5 - 8.0 (β_2-microglobulin is completely denatured within 2 hours at the pH 4.0 and 37°C). As interferences from the urine are deemed possible, dilute urine samples more than 150-fold. As the content of β_2-microglobulin is expected to be in the order of μg in one mL of urine, dilute it whenever deemed necessary.

ex.) Add 2 mL of the sample diluent to 10 μL of urine (201-fold dilution).

6.3 Assay Protocol

Bring all reagents and samples to room temperature before use. It is recommended that all samples, including the standards, are assayed in duplicate.

1) Add 300 μL of the wash buffer to each well of the ELISA plate. Incubate for 10 minutes at room temperature. (no adverse effect, even if left standing for up to 30 minutes.)
2) Aspirate each well to remove the solution.
3) Add 100 μL of the standard rat β_2-microglobulin solution or unknown samples to each well, and incubate for 2 hours at room temperature.
4) Aspirate each well and wash with wash the wells buffer (300 μL/well). Repeat the washing procedure further twice. Complete removal of an aqueous fluid in each wash is essential to good performance.
5) Add 100 μL of the enzyme-labeled antibody solution to each well, and incubate for 1 hour at room temperature.
6) Wash 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 an absorbance at 450 nm (A_{450}) with a microplate reader.

7. Data Calculation

1) Average the duplicate reading for each standard and each sample.
2) Plot the values of A_{450} (Y-axis) versus the concentrations of the standard solutions (X-axis), thus draw a standard curve.
3) Apply an A_{450} value of each sample in the standard curve, so as to read a β_2-microglobulin concentration in it's sample.
4) In case of a diluted, multiply the β_2-microglobulin concentration by the dilution factor to get a β_2-microglobulin concentration in the original sample (serum, plasma and urine).

8. Safety Warnings and Precautions

- Strictly observe the storage condition for each reagent.
- All reagents should be brought to room temperature before use.
- Use reagents after confirming complete dissolution and uniformity.
- Take care not to inflict damage on any well when aspirating an aqueous fluid in each well.
- When measuring many samples in one assay batch, the time period of each reaction for all samples should be uniformed at a fixed time as designated.
- Prepare a standard curve for every measurement.
- Prepare the substrate solution with a clean vessel.
- White powder may sometimes be found in the wells of the ELISA plate. This is due to the dried blocking solution, but will have no effect on the measurement.
- Take care to handle the stop solution, very harmful.

9. Performance Characteristics

9.1 Quantitative Range

0.31 – 20 ng/mL of rat β_2-microglobulin

9.2 Intra - assay Precision

Standards

Rat β_2-microglobulin (ng/mL)	(Replicate)	A_{450} (mean)	C.V. (%)
0	(N=8)	0.078	5.1
0.31	(N=8)	0.148	2.7
0.63	(N=8)	0.229	5.2
1.25	(N=8)	0.371	3.2
2.5	(N=8)	0.636	2.0
5	(N=8)	1.027	2.5
10	(N=8)	1.464	3.6
20	(N=8)	1.809	4.5

Samples

Sample	(Replicate)	β_2-microglobulin conc. (ng/mL)			
		Serum		Urine	
		mean	CV (%)	mean	CV (%)
A	(N=8)	0.73	5.5	0.84	3.6
B	(N=8)	3.40	3.0	3.10	3.2
C	(N=8)	6.60	7.1	11.0	6.3

C.V. = coefficient of variation

Sample A: 5,000-fold dilution of rat serum specimens (male, 7 weeks of age)
Sample B and C: Sample A spiked with standard rat β_2-microglobulin

9.3 Inter-assay Precision

Standards

Rat β_2-microglobulin (ng/mL)	(Replicate)	A_{450} (mean)	C.V. (%)
0	(N=8)	0.064	7.8
0.31	(N=8)	0.222	11.7
0.63	(N=8)	0.337	13.1
1.25	(N=8)	0.501	10.6
2.5	(N=8)	0.726	7.4
5	(N=8)	0.998	8.2
10	(N=8)	1.287	7.3
20	(N=8)	1.570	5.9

Samples

Sample	(Replicate)	β_2-microglobulin conc. (ng/mL)			
		Serum		Urine	
		mean	CV (%)	mean	CV (%)
A	(N=8)	0.83	6.0	0.95	6.3
B	(N=8)	2.50	5.2	3.20	6.7
C	(N=8)	6.40	8.1	12.7	12.7

C.V. = coefficient of variation

Sample A: 5,000-fold dilution of rat serum specimens (male, 7 weeks of age)
Sample B and C: Sample A spiked with standard rat β_2-microglobulin

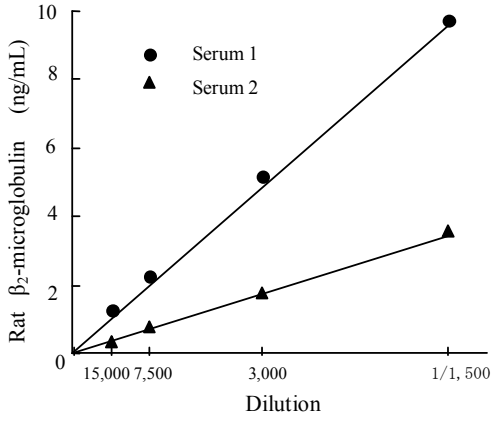
9.4 Recovery

Samples were prepared by spiking rat β_2-microglobulin to a diluted serum (more than 1,500-fold) or to urine (more than 150-fold) of rats (male, 7 weeks of age).

Sample	Spiked amount (ng/mL)	Measured value (ng/mL)	Expected value (ng/mL)	Recovery (%)
Serum 1	0	1.30	-	-
	0.63	2.12	1.93	109.8
	1.25	2.55	2.55	100.0
	2.5	3.66	3.80	96.3
	5.0	6.13	6.20	97.3
	10.0	11.16	11.30	98.8
Urine 1	0	1.60	-	-
	0.63	2.43	2.23	109.0
	1.25	3.07	2.85	107.7
	2.5	4.38	4.10	106.8
	5.0	6.80	6.60	103.0
	10.0	11.06	11.60	95.3
Urine 2	0	0.40	-	-
	0.63	1.05	1.03	101.9
	1.25	1.84	1.65	11.5
	2.5	2.80	2.90	96.6
	5.0	5.38	5.40	99.6
	10.0	10.19	10.40	98.0

9.5 Linearity of Dilution

Samples were prepared by a serial dilution of a rat serum with the sample diluent from 1,500- to 15,000-fold.



10. Storage and Expiry

Store all reagents at 2-10°C under dark and use until a stated expiration date (one year after manufactured).

11. Package

96 tests per kit

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