NOVEL N-ACTTL-β-D-GLUCOSAMINIDASE DETECTION REAGENT

TECHNICAL FIELD

[0001] The present disclosure relates to a novel N-acetyl-β-D-glucosaminidase detection reagent.

BACKGROUND

[0002] N-Acetyl-β-D-glucosaminidase (NAG), also referred to as urease, is an acidic hydrolase that has a molecular weight of 140 kD and exists in cytolysosome, and is mainly present in epithelial cells of proximal convoluted tubule of kidney in a human body. Researches show that NAG is sensitive to renal tubule injury response, which can more rapidly and directly reflect the degree and condition change of renal tubule activity injury compared with other low-molecule-weight proteins in urine, however, a few amount of NAG is contained in urine of healthy people. In addition, using urine as a specimen is also a non-invasive examination, which is in line with the trend of modern medical examination methods and suitable for daily examination and continuous dynamic analysis. Accordingly, determination of activity of NAG in urine has an important clinical application value on early detection of renal tubule injury caused by diabetes, various renal parenchymal diseases, drug and heavy metal renal toxicity, urinary tract infection (pyelonephritis), renal allograft rejection, hypertension, autoimmune diseases and the like

[0003] At present, NAG activity detection methods include radioimmunoassay, fluorescence analysis and visible spectrophotometry. Among them, instruments required for radioimmunoassay and fluorescence analysis are expensive in price, and have high requirements on preparation of a substrate solution, thus are not suitable for large-flow automated analysis in clinic; whereas, in the reported spectrophotometry, there are problems such as poor substrate solubility or poor reagent stability, and therefore the spectrophotometry is difficult to meet the requirements of clinical tests.

SUMMARY

[0004] In view of the defects in the prior art, the present disclosure provides a detection reagent with good stability, high sensitivity, no re-dissolution and direct use and a detection method.

[0005] Provided is a urine N-acetyl-β-D-glucosaminidase detection reagent, the detection reagent consisting of two reagents: reagent R1 and reagent R2, wherein:

[0006] the reagent R1 comprises components having the following concentrations:

Citrate buffer solution	150-300 mmol/L
Substrate	1-5 mmol/L
BSA	3-5 g/L
Magnesium chloride	0.5-5 g/L
EDTA·2Na	0.5-5 g/L
Trixon-X100	2-5 ml/L
SDS	0.5-5 g/L
Sodium sulfite	0.01-1 g/L
Polyethylene glycol 6000	0.01-2 g/L
Ethylene glycol	0.5-3 ml/L
glucose	5-30 g/L
β-cyclodextrin	0.1-3 g/L
Crown 18 ether	0.1-3 g/L
Preservative	0.1-3 g/L

[0007] The reagent R2 comprises components having the following concentrations:

Carbonate buffer solution	150-500mmol/L
Tween 20	1-10 ml/L
Urea	1.5-5 g/L
BSA	0.1-10 g/L
Preservative	0.1-3 g/L

[0008] Preferably, the substrate is 5-[4-(3-methoxy-phenylmethene)]-raotannine-3-ammonium acetate N-acetylamino-β-D-glucoside (VRA-NAG).

[0009] Further, the BSA is bovine serum albumin, SDS is sodium dodecyl sulfate, EDTA·2Na is disodium ethylenediamine tetraacetic acid.

[0010] Preferably, the preservative is one of sodium azide, NaN_3 and methyl p-hydroxybenzoate.

[0011] Further, in the urine N-acetyl-β-D-glucosaminidase detection reagent, the R1 buffer solution is a citrate buffer solution being at 15-30℃ and at the pH of 4.0-6.0.

[0012] Further, in the urine N-acetyl-β-D-glucosaminidase detection reagent, the R2

buffer solution is a carbonate buffer solution being at 15-30℃ and at the pH of 11.0-12.0.

[0013] Further, the detection method for detecting the urine N-acetyl-β-D-glucosaminidase with the urine N-acetyl-β-D-glucosaminidase detection reagent conducts determination by using a fully automatic biochemical analyzer with a main detection wavelength of 505 nm by virtue of an end point method.

[0014] Further, a ratio of reagent R1 to reagent R2 is 3:1.

[0015] The basic principle of the present disclosure is as follows:

[0016] The N-acetyl-β-D-glucosaminidase in urine catalyzes the hydrolysis of 5-[4-(3-methoxy-phenylmethene)]-raotannine-3-ammonium acetate N-acetylamino-β-D-glucoside (VRA-NAG) in the reagent into 5-[4-(3-methoxy-phenylmethene)]-raotannine-3-ammonium acetate (VRA) in an acidic environment, subsequently conformation of VRA changes in a strong alkaline environment to produce fluorescence, the solution exhibits purple red, there is an absorption peak at the wavelength of 505 nm, and the activity of N-acetyl-β-D- glucosaminidase can be determined by detecting the change in absorbance at the wavelength of 505 nm.

[0017] VRA-NAG + H_2O $\xrightarrow{\text{NAG}}$ VRA

[0018] Note: VRA-NAG: 5-[4-(3-methoxy-phenylmethene)]-rotannine-3-ammonium acetate N-acetylamino-β-D-glucoside, with a molecular weight of 545.

[0019] NAG: N-acetyl-β-D- glucosaminidase

[0020] VRA: 5-[4-(3-methoxy-phenylmethene)]-raotannine-3-ammonium acetate

[0021] The present disclosure has the beneficial effects:

[0022] 1) new 5-[4-(3-methoxy-phenylmethene)]-raotannine-3-ammonium acetate-N-acetylamino-β-D-glucoside (VRA-NAG) is used as a reaction substrate, which not only solves the problem of large reagent chromogen interference, but also significantly enhances the solubility of the substrate and the accuracy of the reagent;

[0023] 2) in the present disclosure, the magnesium ion is used as a substrate chelating agent for the first time, and the magnesium ion is chelated with VRA-NAG with a cyclic structure to form a hexatomic ring chelate, thereby enhancing the stability of the reagent;

[0024] 3) the reagent is good in accuracy and stability, strong in anti-interference performance and convenient to use, and meets the demand of clinical batch detection.

BRIEF DESCRIPTION OF THE DRAWINGS

[0025] Fig.1 is a relevant curve graph of an N-acetyl-β-D-glucosaminidase detection reagent in example 1 of the present disclosure;

[0026] Fig.2 is a relevant curve graph of an N-acetyl-β-D-glucosaminidase detection reagent in example 2 of the present disclosure;

[0027] Fig.3 is a relevant curve graph of an N-acetyl-β-D-glucosaminidase detection reagent in example 3 of the present disclosure;

[0028] Fig.4 is a stability curve graph of N-acetyl-β-D-glucosaminidase detection reagents in examples 1-3 and comparative examples of the present disclosure within the expiration date.

DETAILED DESCRIPTION OF THE EMBODIMENTS

[0029] For making the technical problems, technical solutions and advantages of the present disclosure more clear, the present disclosure will be described in detail in combination with specific embodiments and drawings.

[0030] Example 1

[0031] An N-acetyl-β-D- glucosaminidase detection reagent in this example comprises reagent R1 and reagent R2, wherein:

[0032] The components and contents of the reagent R1 are as follows:

Citrate buffer solution (pH=4.0, 15℃)	200 mmol/L
VRA-NAG	3 mmol/L
BSA	5 g/L
Magnesium chloride	1 g/L
EDTA·2Na	2 g/L
Trixon-X100	3 ml/L
SDS	1 g/L
Sodium sulfite	0.1 g/L
Polyethylene glycol 6000	0.5 g/L
Ethylene glycol	0.9 ml/L
Glucose	20 g/L
β-cyclodextrin	2 g/L
Crown 18 ether	2 g/L
$NaNO_3$	1 g/L

[0033] The components and contents of the reagent R2 are as follows:

Carbonate buffer solution (pH=11，15℃)	200 mmol/L
Tween 20	2 ml/L
Urea	3 g/L
BSA	1 g/L
$NaNO_3$	0.5 g/L

[0034] The use method of this detection reagent:

[0035] The N-acetyl-β-D-glucosaminidase detection reagent described in this example is used for detection via a full-automatic biochemical analyzer with a double-reagent function such as Hitachi 7180 full-automatic analyzer, Hitachi 7080 full-automatic analyzer and Mindray 800, and an end point method is utilized for determination, and a main detection wavelength is set as 505 nm. The detection reagents R1 and R2 are placed on corresponding reagent positions in a ratio of 3:1, double distilled water, a calibrator, quality control and a sample to be tested are placed on a sample tray. Operations are shown in Table 1.

Table 1 Reagent detection method in example 1

Solution added	Blank tube	Standard tube 1	Standard tube 2	Test tube
Reagent R1	180 μL	180 μL	180 μL	180 μL
Distilled water	6 μL	–	–	–
Calibrator	–	6 μL	–	–
Quality control products	–	–	6 μL	–
To-be-detected samples	–	–	–	6 μL
Mix, pre-incubate for 5 min at a constant temperature of 37℃, and record absorbance A1				

Reagent R2	60 μL	60 μL	60 μL	60 μL
Evenly mix, react for 5 min, then record absorbance A2 at a main wavelength of 505 nm, and calculate ΔA				

[0036] Calculation formula: concentration of urine N-acetyl-β-D-glucosaminidase (U/L) = (sample ΔA ÷ calibrator ΔA) × concentration of calibrator.

[0037] **Example 2**

[0038] An N-acetyl-β-D-glucosaminidase detection reagent in this example comprises reagent R1 and reagent R2, wherein:

[0039] The components and contents of the reagent R1 are as follows:

Citrate buffer solution (pH=5.0, 20℃)	150 mmol/L
VRA-NAG	1 mmol/L
BSA	3 g/L
Magnesium chloride	0.5 g/L
EDTA·2Na	0.5 g/L
Trixon-X100	2 ml/L
SDS	0.5 g/L
Sodium sulfite	0.01 g/L
Polyethylene glycol 6000	0.01 g/L
Ethylene glycol	0.5 ml/L
Glucose	5 g/L
β-cycldextrin	0.1 g/L
Crow 18 ether	0.1 g/L
Sodium azide	0.1 g/L

[0040] The components and contents of the reagent R2 are as follows:

Carbonate buffer solution (pH=11.5, 20℃)	150 mmol/L
Tween 20	1 ml/L
Urea	1.5 g/L
BSA	0.1 g/L
Sodium azide	0.1 g/L

[0041] The use method and detection method are the same as those in example 1.

[0042] Example 3

[0043] An N-acetyl-β-D-glucosaminidase detection reagent in this example comprises reagent R1 and reagent R2, wherein:

[0044] The components and contents of the reagent R1 are as follows:

Citrate buffer solution (pH=6.0, 25℃)	300 mmol/L
VRA-NAG	5 mmol/L
BSA	5 g/L
Magnesium chloride	5 g/L
EDTA·2Na	5 g/L
Trixon-X100	5 ml/L
SDS	5 g/L
Sodium sulfite	1 g/L
Polyethylene glycol 6000	2 g/L
Ethylene glycol	3 ml/L
Glucose	30 g/L
β-cyclodextrin	3 g/L
Crown 18 ether	3 g/L
Methyl p-hydroxybenzoate	3 g/L

[0045] The components and contents of the reagent R2 are as follows:

Carbonate buffer solution (pH=11.5, 25℃)	500 mmol/L
Tween 20	10 ml/L
Urea	5 g/L
BSA	10 g/L
Methyl p-hydroxybenzoate	3 g/L

[0046] The use method and detection method are the same as those in example 1.

[0047] Example 4

[0048] Interference experiment: freshly mixed urine was divided into 2 equal parts, then each part of urine was divided into 6 equal parts, and then different interferents were added, so that the concentration in urine reached the requirements in Table 2. Then, the

reagents obtained in this example together with urine N-acetyl-β-D-glucosaminidase detection reagent that is common and recognized on the market as control group were used to detect the content of NAG in urine. The determination results of control reagent group and determination results of groups with addition of different interferents are seen in Table 2.

[0049] Relative deviation (%) = (average determination value of interfering sample-average determination value of control sample)/average determination value of control sample × 100%.

Table 2 Comparison of anti-interference performance of example reagents

Types of interferents	Concentrations of interferents	Example 1		Example 2		Example 3		Control group	
		NAG average value (U/L)	Relative deviation	NAG average value (U/L)	Relative deviation	NAG average value (U/L)	Relative deviation	NAG average value (U/L)	Relative deviation
No interferent (blank group)	0	11.3	0.00	11.3	0.00	11.5	0.00	11.5	0.00
Glucose (mmol/L)	20	11.2	-0.88%	11.7	3.54 %	11.8	2.61 %	12.3	5.09 %
Bilirubin	40	11.5	1.77%	11.9	5.31 %	11.8	2.61 %	12.1	6.96 %
Hemoglobin	200	11.0	-2.65%	11.6	2.65 %	10.9	- 5.22 %	10.4	- 9.57 %
Trigluceride	500	11.5	1.77%	11.7	3.54	11.7	1.74	10.9	-

					%		%		5.22 %
Ascorbic acid (mg/dL)	50	11.3	0.00%	12.0	6.19 %	11.0	-4.35 %	12.6	9.57 %

[0050] It can be seen from Table 2 that the detection results of the detection reagents in examples 1-3 are not obviously interfered when Glucose ≤ 20mmol/L, bilirubin ≤ 40mg/dL, hemoglobin ≤ 200mg/dL, triglyceride ≤ 500mg/dL and ascorbic acid ≤ 50mg/dL. In the presence of interferents having the above concentrations, the detection results of the reagents in the control group are obviously interfered, which indicates that the anti-interference ability of the detection reagent can be effectively enhanced by using new substrate 5-[4-(3-methoxy-phenylmethene)]-raotannine-3-ammonium acetate N-acetylamino-β-D-glucoside (VRA-NAG) as a reaction substrate and using magnesium ion as a substrate chelating agent to avoid that interference from glucose, bilirubin and ascorbic acid. The anti-interference performance of the reagent in example group is significantly improved, which is far superior to that in control reagent.

[0051] Example 5

[0052] Correlation experiment: the reagent formula described in this example is used to prepare the detection reagent, and then the prepared detection reagent is compared with the N-acetyl-β-D-glucosaminidase detection reagent that is common on the market, recognized by the State Food and Drug Administration and purchased from a company, 40 clinical urine samples are used in each example group, and detection results are as shown in Table 3. Meanwhile, a correlation curve between each example group and control group is obtained (as shown in Fig.1). The detection results show that a correlation coefficient between example 1 and control group is 0.9979, a correlation coefficient between example 2 and control group is 0.9949, a correlation coefficient between example 3 and control group is 0.9962, and relative deviations of 40 samples in each example group and control group were all ≤ 10%, indicating that there is a great correlation between examples 1-3 and control group.

Table 3 Comparison detection results of example reagents and urine n-acetyl-β-D-glucosaminidase detection kit that is common and recognized on the market

Sample number	Example 1 (U/L)	Example 2 (U/L)	Example 3 (U/L)	Control group (U/L)	Relative deviation
1	2.10	2.05	2.14	2.15	2.38%
2	0.50	0.5	0.61	0.53	6.00%
3	1.55	1.59	1.63	1.50	-3.23%
4	2.34	2.07	2.2	2.15	-8.12%
5	2.64	2.35	2.51	2.65	0.38%
6	1.20	1.04	1.23	1.15	-4.17%
7	7.56	7.21	7.78	7.75	2.51%
8	7.91	7.33	8.22	7.85	-0.76%
9	0.31	0.3	0.33	0.33	6.45%
10	0.85	0.79	0.87	0.80	-5.88%
11	0.75	0.72	0.88	0.80	6.67%
12	0.57	0.55	0.59	0.60	5.26%
13	6.55	6.17	6.79	6.66	1.68%
14	2.45	2.22	2.56	2.30	-6.12%
15	1.73	1.59	1.87	1.73	0.00%
16	5.66	5.51	5.74	5.55	-1.94%
17	0.96	0.91	1.11	1.02	6.25%
18	6.52	6.22	6.84	6.35	-2.61%
19	2.10	1.97	2.31	2.00	-4.76%
20	1.50	1.48	1.58	1.50	0.00%
21	2.64	2.56	2.9	2.81	6.44%
22	1.30	1.16	1.40	1.18	-9.23%
23	1.50	1.32	1.67	1.45	-3.33%
24	10.01	9.8	10.07	9.85	-1.60%

25	3.80	2.4	3.82	3.50	-7.89%
26	2.15	2.11	2.31	2.22	3.26%
27	1.40	1.19	1.55	1.30	-7.14%
28	5.70	5.57	5.68	5.55	-2.63%
29	10.04	9.44	10.98	9.78	-2.59%
30	1.50	1.31	1.57	1.50	0.00%
31	3.15	2.99	3.59	3.35	6.35%
32	1.20	1.1	1.35	1.30	8.33%
33	2.70	2.5	2.86	2.57	-4.81%
34	6.95	6.66	7.24	6.70	-3.60%
35	9.06	8.54	9.38	8.80	-2.87%
36	4.50	4.31	4.87	4.35	-3.33%
37	2.60	2.38	3.19	2.80	7.69%
38	0.90	0.9	0.92	0.91	1.11%
39	1.00	0.97	1.22	1.10	10.00%
40	8.67	8.51	8.97	8.80	2.92%
Correlation	0.9979	0.9949	0.9962	/	/

[0053] Example 6

[0054] Stability comparison test of reagent: each group of reagents in examples 1-3 were respectively subpackaged into 13 groups, the subpackage amount of each group: 18 mL of reagent R1, 6 mL of reagent R2; and 13 groups of N-acetyl-β-D-glucosaminidase detection kits that were common on the market, recognized by State Food and Drug Administration and purchased from a company were used as control. The reagents were put in a refrigerator at 2-8℃, a group of reagents were taken out on the same day of each month to test NAG quality control (a target value was 25.8 μmol/L). The detection results are shown in Fig. 2. The reagents in examples 1-3 are more stable than N-acetyl-β-D-glucosaminidase detection kit that is common on the market under the storage condition of 2-8 ℃.

[0055] By verification, this reagent has a good correlation with similar detection reagents,

and the results of clinical detection samples are consistent. This reagent can meet the market requirements for product application, and has good anti-interference performance, and is a more stable and good N-acetyl-β-D-glucosaminidase detection reagent.