（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K757-M

Specification：48T (32 samples)/96T (80 samples)

Measuring instrument：Microplate reader(530-550 nm)

Detection range：0.036-1.0 mg/mL

Elabscience® Revertose Colorimetric Assay Kit (DNS Method)

This manual must be read attentively and completely before using this product.

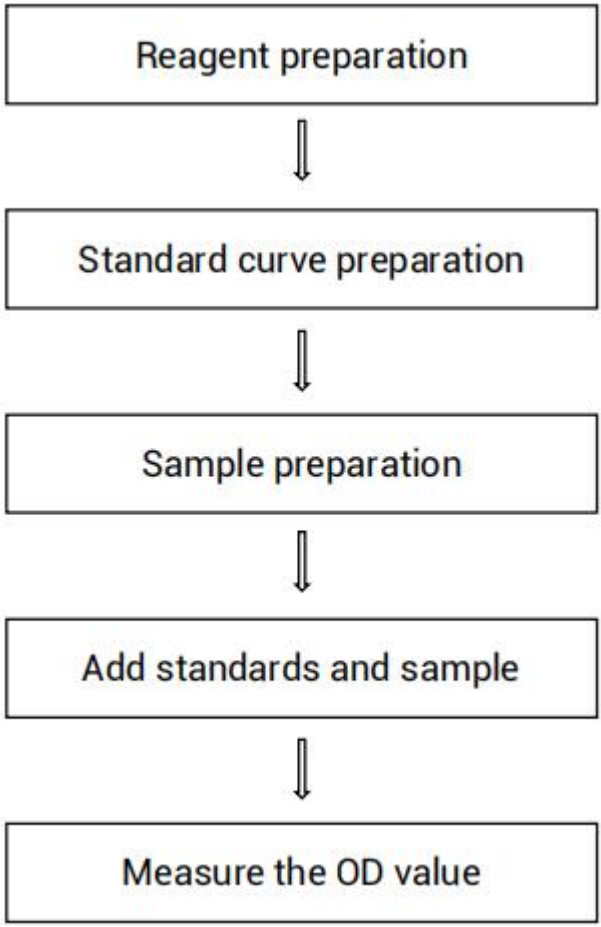
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Table of contents

Assay summary



Intended use

This kit can be used to measure revertose content in in serum, plasma, animal and plant tissue sample.

Detection principle

Revertose are sugars with reducing properties. In sugars, both monosaccharides containing free aldehyde or ketone groups and disaccharides containing free aldehyde groups are reductive. Revertose mainly include glucose, fructose, galactose, lactose, maltose, etc. Revertose are reduced to amino compounds after co-heating with 3, 5-dinitrosalicylic acid under alkaline conditions, and are reddish brown in alkaline solution. The amount of revertose is proportional to the depth of color of reddish-brown substances, so as to determine the content of revertose in samples.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Chromogenic Agent	2 mL × 1 vial	4 mL × 1 vial	2-8℃, 12 months, shading light
Reagent 2	Standard	Powder × 1 vial	Powder × 1 vial	2-8℃, 12 months
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces		
	Sample Layout Sheet	1 piece		

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Microplate reader (530-550 nm, optimum wavelength: 540 nm), Water bath

Reagent preparation

① Equilibrate all the reagents to 25℃ before use.

② Before sample detection, the chromogenic agent was heated in a 75°C water bath for 10 min and cooled to 25°C with running water before use.

③ The preparation of 10 mg/mL standard solution:
Dissolve one vial of standard with 1 mL of double distilled water, mix well to dissolve. Store at 2-8°C for a month.

④ The preparation of 1 mg/mL standard solution:
Before testing, please prepare sufficient 1 mg/mL standard solution. For example, prepare 1000 μL of 1 mg/mL standard solution (mix well 100 μL of 10 mg/mL standard solution and 900 μL of double distilled water). Store at
2-8°C for 2 weeks.

⑤ The preparation of standard curve：
Always prepare a fresh set of standards. Discard working standard dilutions after use.
Dilute 1 mg/mL standard with double distilled water to a serial concentration, the recommended dilution gradient is 0, 0.1, 0.2, 0.3, 0.4, 0.6, 0.8, 1.0 mg/mL. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mg/mL)	**0**	**0.1**	**0.2**	**0.3**	**0.4**	**0.6**	**0.8**	**1.0**
1 mg/mL standard (μL)	0	20	40	60	80	120	160	200
Double distilled water (μL)	200	180	160	140	120	80	40	0

Sample preparation

① Sample preparation

Serum or plasma samples: detect directly.

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Homogenize 20 mg tissue in 180 μL double distilled water with a dounce homogenizer at 4℃.

③ Centrifuge at 10000 × g for 10 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

④ Meanwhile, determine the protein concentration of supernatant (animal tissue: E-BC-K318-M; plant tissue: E-BC-K168-M).

② Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
Human serum	1
10% Mouse liver tissue homogenate	3-10
10% Wheat tissue homogenate	15-30
10% Paddy tissue homogenate	15-30
10% Corn tissue homogenate	15-30
10% Pumpkin tissue homogenate	10-20

Note: The diluent is double distilled water. For the dilution of other sample types, please do pretest to confirm the dilution factor.

Operating steps

① Standard well: add 30 μL of standard with different concentrations into 0.5 mL EP tubes.

Sample well: add 30 μL of sample into 0.5 mL EP tubes.

② Add 30 μL of chromogenic agent into each tube.

③ Mix fully and heated in a 95°C water bath for 10 min and cooled with running water.

④ Add 180 μL of double distilled water into each tube.

⑤ Mix fully and take 200 μL solution of each tube to the microplate. Measure the OD values of each well at 540 nm with microplate reader.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard # ①) from all standard readings. This is the absoluted OD value.

3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

1. Serum and plasma samples:

$$\text{revertose content (mmol/L)} = \frac{\Delta A - b}{a} \times f \div 180^{*} \times 1000^{**}$$

2. Tissue samples:

$$\text{revertose content (mmol/kg wet weight)} = \frac{\Delta A - b}{a} \times V \div m \times f \div 180^{*} \times 1000^{**}$$

or

$$\text{revertose content (mmol/gprot)} = \frac{\Delta A - b}{a} \div C_{pr} \times f \div 180^{*} \times 1000^{**}$$

[Note]

ΔA: $OD_{sample} - OD_{blank}$.

180*: The molecular weight of glucose, 180 mg/mmol.

1000**: 1 mL = 10^-3 L； 1 g = 10^-3 kg； 1 mgprot = 10^-3 gprot.

V: The volume of double distilled water in the preparation step of tissue, mL.

m: The wet of sample, g

f: Dilution factor of sample before test.

C_{pr}: Concentration of protein in sample, mgprot/mL.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Parameters	Sample 1	Sample 2	Sample 3
Mean (mg/mL)	0.5	0.7	0.9
%CV	1.0	1.9	2.9

Inter-assay Precision

Three human serum were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (mg/mL)	0.25	0.50	0.75
%CV	2.1	4.5	5.3

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 104%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (mg/mL)	0.5	0.7	0.9
Observed Conc. (mg/mL)	0.52	0.74	0.93
recovery rate(%)	103.0	105.8	103.8

Sensitivity

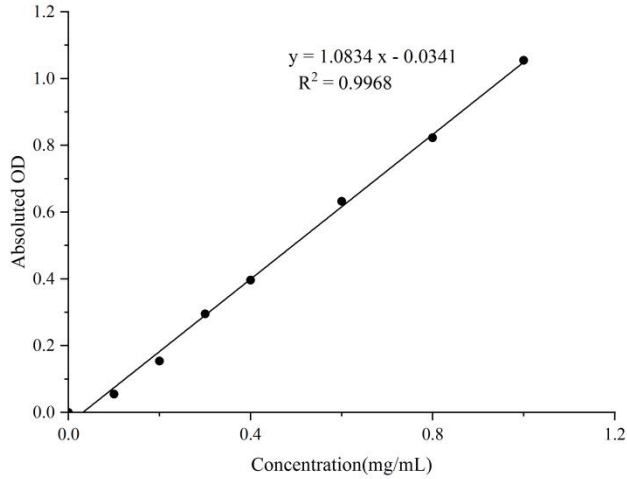
The analytical sensitivity of the assay is 0.036 mg/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the

corresponding concentration.

2. Standard curve:

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only：

Concentration (mg/mL)	0	0.1	0.2	0.3	0.4	0.6	0.8	1.0
OD	0.055	0.109	0.215	0.352	0.458	0.675	0.882	1.104
	0.055	0.111	0.203	0.349	0.444	0.700	0.874	1.115
Average OD	0.055	0.110	0.209	0.351	0.451	0.688	0.878	1.110
Absolute OD	0.000	0.055	0.154	0.296	0.396	0.633	0.823	1.055



Appendix Π Example Analysis

Example analysis：

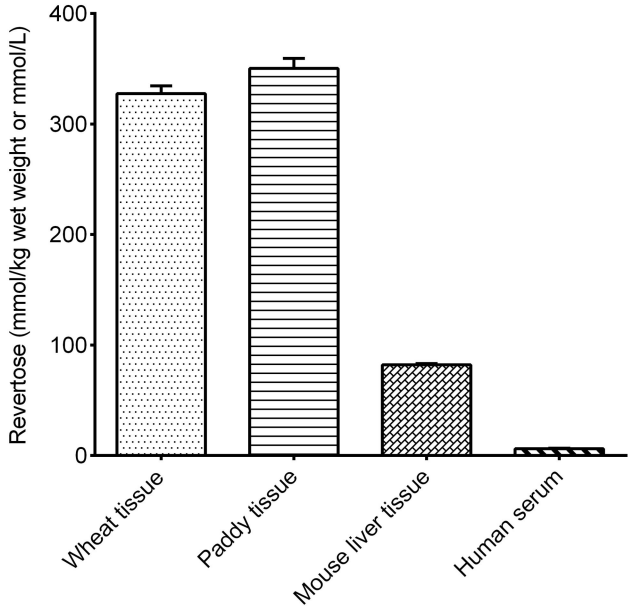
Take 30 μL of 10% wheat tissue homogenate (dilute for 25 times) and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 1.0834 x - 0.0341, the OD value of the sample well is 0.309, the OD value of the blank well is 0.055, and the calculation result is:

revertose content (mmol/kg wet weight) = (0.309 - 0.055 + 0.0341) ÷ 1.0834 × 0.9

÷ 0.1 × 25 ÷ 180 × 1000 = 332.38 mmol/kg wet weight

Detect 10% wheat tissue homogenate (dilute for 25 times), 10% paddy tissue homogenate (dilute for 25 times), 10% mouse liver tissue homogenate (dilute for 5 times) and human serum, according to the protocol, the result is as follows：



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.