TECHNICAL MANUAL

Revertose Colorimetric Assay Kit (DNS Method)

- **SKU CODE:** MAES0517
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Add standards and sample
- Measure the absorbance value

2. Intended use

This kit can be used to measure reducing sugar content in serum, plasma, animal and plant tissue samples.

3. Detection principle

Reducing sugars are sugars with reducing properties. In sugars, both monosaccharides containing free aldehyde or ketone groups and disaccharides containing free aldehyde groups are reductive. Reducing sugars mainly include glucose, fructose, galactose, lactose, maltose, etc.

Detection principle: Reducing sugars are converted to amino compounds after co-heating with 3,5-dinitrosalicylic acid under alkaline conditions, and appear reddish brown in alkaline solution. The amount of reducing sugars is proportional to the intensity of the reddish-brown color, allowing determination of reducing sugar content in samples.

4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Chromogenic Agent	2 mL x 1 vial	4 mL x 1 vial	2-8 °C, 12 months, shading light
Reagent 2	Standard	Powder x 1 vial	Powder x 1 vial	2-8 °C, 12 months
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces	2 pieces	

5. Materials prepared by users

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

6. Reagent preparation

1. Equilibrate all reagents to 25 °C before use.
2. Before sample detection, heat the chromogenic agent in a 75 °C water bath for 10 min and cool to 25 °C with running water before use.
3. **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. **Preparation of 1 mg/mL standard solution:** Before testing, prepare sufficient 1 mg/mL standard solution. For example, to prepare 1000 µL of 1 mg/mL standard solution, thoroughly mix 100 µL of 10 mg/mL standard solution and 900 µL of double distilled water. Store at 2-8 °C for 2 weeks.
5. **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 serial concentrations. The recommended dilution gradient is 0, 0.1, 0.2, 0.3, 0.4, 0.6, 0.8, 1.0 mg/mL.

7. 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 °C. 3. Centrifuge at 10,000 x g for 10 min at 4 °C to remove insoluble material. Collect supernatant and keep it on ice for detection. 4. Meanwhile, determine the protein concentration of supernatant (animal tissue: MAES0177; plant tissue: MAES0126).
2. **Sample dilution:** The recommended dilution factor for different samples is as follows (for reference only): 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 dilution of other sample types, please perform a pretest to confirm the dilution factor.

8. 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 heat in a 95 °C water bath for 10 min and cool with running water.
4. Add 180 µL of double distilled water into each tube.
5. Mix fully and transfer 200 µL solution from each tube to the microplate. Measure the OD values of each well at 540 nm with microplate reader.

9. 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 absolute OD value.

3. Plot the standard curve using absolute OD value of standard and corresponding 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:

reducing sugar content (mmol/L) = (ΔA - b)/a × f ÷ 180* × 1000**

2. Tissue samples:

reducing sugar content (mmol/kg wet weight) = (ΔA - b)/a × V ÷ m × f ÷ 180* × 1000**

or

reducing sugar content (mmol/g_{prot}) = (ΔA - b)/a ÷ C_{pr} × f ÷ 180* × 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 mg_{prot} = 10^{-3} g_{prot}

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

m: The weight of sample, g

f: Dilution factor of sample before test

C_{pr}: Concentration of protein in sample, mg_{prot}/mL

10. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

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

Intra-assay Precision

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 samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Inter-assay Precision

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

Recovery

Three samples of high, middle, and low concentrations were tested in 6 replicates for each concentration to obtain an average recovery rate of 104%.

Recovery

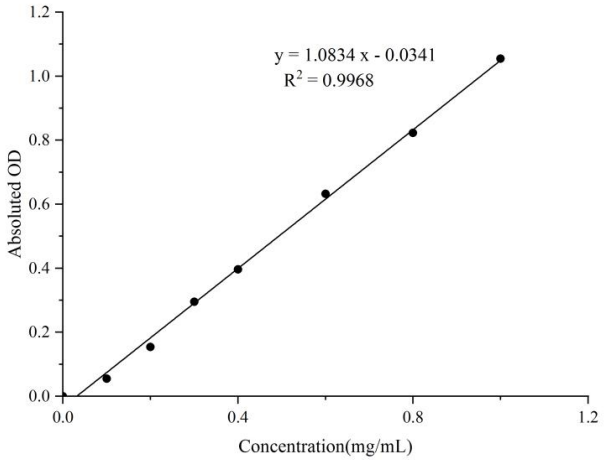
	Sample 1	Sample 2	Sample 3
Expected Conc. (mg/mL)	0.5	0.7	0.9
Observed Conc. (mg/mL)	0.52	0.74	0.93
Recovery rate (%)	103.0	105.8	103.8

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.

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), the standard curve and data are provided below for reference only:



Standard curve

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

Concentration (mg/mL)	OD		Average OD	Absolute OD
1.0	1.104	1.115	1.110	1.055

11. Appendix II Example Analysis

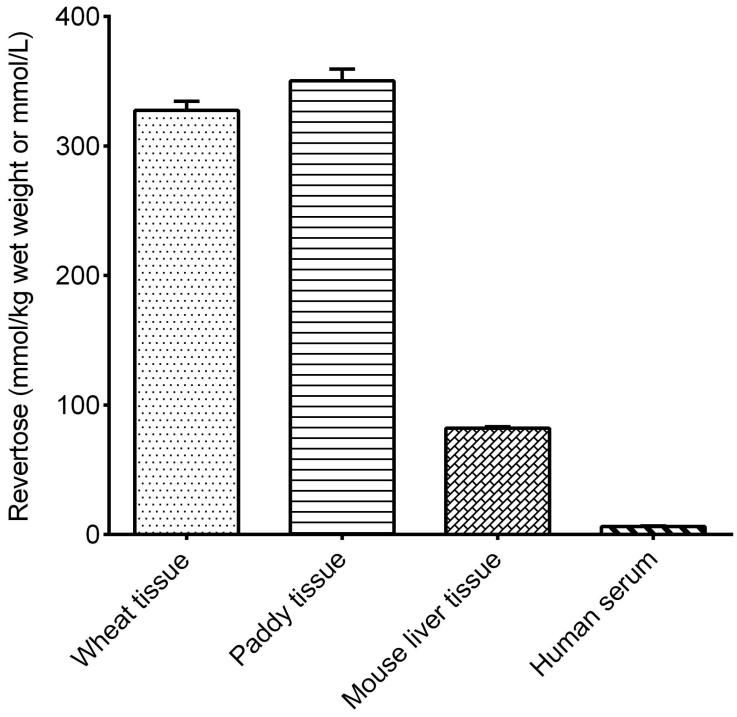
Example analysis:

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

Standard curve: y = 1.0834x - 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:

reducing sugar 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 (diluted 25 times), 10% paddy tissue homogenate (diluted 25 times), 10% mouse liver tissue homogenate (diluted 5 times) and human serum according to the protocol. The results are as follows:



12. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.
2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.
3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.
4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.
5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.
6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.