Reducing Sugar (RS) Assay Kit (Colorimetric)

Cat #: orb1173187 (manual)

Product Features

Product name: Reducing Sugar (RS) Assay Kit (Colorimetric)

Catalog number: orb1173187

Sample Types: Plant Tissues, Animal Tissues, Cells, Bacteria, Serum, Plasma

Detection range: 0.05-0.6 mg/mL

Sensitivity: 0.025 mg/mL

Storage: Stored at 4℃ for 12 months, protected from light.

Assay Principle

Reducing sugars (RS) are widely present in animals, plants, microorganisms, and cultured cells. In plants, major RS includes glucose, fructose, and maltose. Glucose and fructose are key respiratory substrates and precursors for sucrose, starch, and cellulose synthesis.

The Reducing Sugar (RS) Assay Kit (Colorimetric) measures RS levels in liquid samples such as tissue homogenates, bacteria, cells, and serum or plasma. The assay is based on the reduction of 3,5-dinitrosalicylic acid to brown-red amino compounds under alkaline conditions, producing a characteristic absorbance at 540 nm. Within a defined range, RS concentration is linearly proportional to absorbance, allowing quantification using a standard curve.

Kit Components

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	60 mL	120 mL	4℃
DNS Reagent	10 mL	20 mL	4℃, protected from light
Standard	Powder×1 vial	Powder×1 vial	4℃

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 540 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Centrifuge, water bath
- Deionized water
- Homogenizer (for tissue samples)

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Sample Preparation

1. Plant or animal tissue samples: Weigh 0.1 g tissues and add 1 mL Extraction Buffer. Homogenize on ice. Incubate in 80℃ for 40 min (cover tightly to prevent moisture evaporation), shake test tube every 5 min, mix well. Centrifuge at 8,000 g for 10 min at 25℃. Use supernatant for assay.

2. Bacteria or cells: Collect bacteria or cells into a centrifuge tube. Then discard the supernatant after centrifugation. Add 1 mL Extraction Buffer for every 5×10^6 bacteria or cells. Ultrasonically break bacteria or cells 5 min (power 20%, work 3 s, intermittent 10 s, work 30 times). Incubate in 80℃ for 40 min (cover tightly to prevent moisture evaporation), shake test tube every 5 min, mix well. Centrifuge at 8,000 g for 10 min at 25℃. Use supernatant for assay.

3. Serum or plasma sample: Take 0.1 mL sample and add 0.9 mL Extraction Buffer, mix well. Incubate in 80℃ for 40 min (cover tightly to prevent moisture evaporation), shake test tube every 5 min, mix well. Centrifuge at 8,000 g for 10 min at 25 ℃. Use supernatant for assay.

Note: 1. It will be better to quantify the total protein with Protein Quantification Kit (BCA Assay), Cat #: orb1147876, if the content is calculated by protein concentration.

2. Extraction Buffer contains components that denature the protein. If the Extraction Buffer is calculated by the protein concentration, the protein needs to be extracted again for determination.

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

DNS Reagent: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C, protected from light.

Note: Extraction Buffer or DNS Reagent has certain irritation, so personal protection is recommended during use.

Standard: Before use, add 1 mL deionized water to the Standard powder and mix well to prepare 10 mg/mL Standard. Storage at 4°C for 2 weeks.

Setting of standard curves: Dilute 10 mg/mL Standard to 0.6, 0.5, 0.4, 0.3, 0.2, 0.1, 0.05 mg/mL with deionized water.

Num.	Volume of 10 mg/mL Standard (µL)	Volume of Deionized Water (µL)	Concentration (mg/mL)
Std.1	60	940	0.6
Std.2	50	950	0.5
Std.3	40	960	0.4
Std.4	30	970	0.3
Std.5	20	980	0.2
Std.6	10	990	0.1
Std.7	5	995	0.05

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 540 nm, visible spectrophotometer was returned to zero with deionized water.

2. Add the following reagents respectively into each tube:

Reagent	Blank Tube (μL)	Standard Tube (μL)	Test Tube (μL)	Control Tube (μL)
Sample	0	0	175	175
Different Concentration Std.	0	175	0	0
Deionized Water	175	0	0	125
Mix well, boiling 5 min (cover tightly to prevent water evaporation), cool immediately to room temperature. Add 200 uL to 96-well plate or microglass cuvette. Measure absorbance of 540 nm in a microplate reader. Calculate $\Delta A_{Test}=A_{Test}-A_{Blank}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.				

Note: The Blank Well and the Standard Well only need to be done 1-2 times. To guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA_{Test} is less than 0.04, increase the sample quantity appropriately. If ΔA_{Test} is larger than 0.6 mg/mL of $\Delta A_{Standard}$, the sample can be appropriately diluted with Extraction Buffer, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.

Calculation of Results

We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1. Drawing of standard curve

With the concentration of the standard solution as the y axis and the $\Delta A_{Standard}$ as the x axis, draw the standard curve. Substitute the ΔA_{Test} into the equation to obtain the y value.

2. Calculating the content of RS

(1) Based on sample quality

$RS\ (\mu g/g) = 1{,}000\times y\times V_{Extraction}\div W\times n = 1{,}000\times y\div W\times n$

(2) According to sample protein concentration

$RS\ (\mu g/mg) = 1{,}000\times y\times V_{Extraction}\div (V_{Extraction}\times Cpr)\times n = 1{,}000\times y\div Cpr\times n$

(3) By the number of bacteria or cells

$RS\ (\mu g/10^4) = 1{,}000\times y\times V_{Extraction}\div 500\times n = 2\times y\times n$

(4) By the volume of serum (plasma)

$RS\ (\mu g/mL) = 1{,}000\times y\times V_{Extraction}\div V_{Sample}\times n = 10{,}000\times y\times n$

Where: 1,000, 1 mg/mL=1,000 μg/mL; $V_{Extraction}$, the volume of Extraction Buffer, 1 mL; V_{Sample}, volume of added serum or plasma, 0.1 mL; Cpr, sample protein concentration, mg/mL; W, sample mass, g; 500, the number of bacteria or cells; n, the sample dilution factor.

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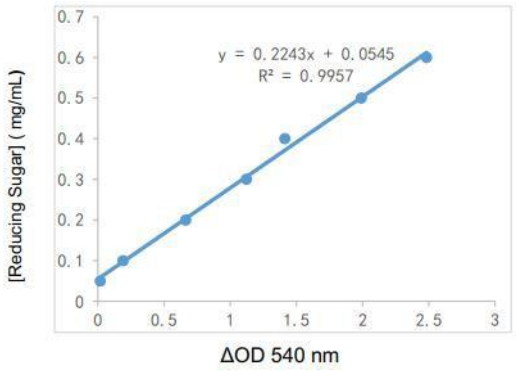
Typical Data

Typical standard curve, for reference only.



Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.

Safety Notes

For your safety and health, please wear a lab coat and wear disposable gloves.

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