

Reducing Sugar Content Assay Kit (DNS, Micro Method)

Cat. No.: R1501790 | Pack size: 48T, 96T | Storage: 2-8°C, protected from light

Specifications

Property	Information
Specifications & Purity	BioReagent
Grade	BioReagent
Application	Cell Metabolism
Sensitivity	Light-sensitive

Storage and Shipping

Property	Information
Storage	Store at 2-8°C, Protected from light
Shipped In	Wet ice
Stability And Storage	Store at 2-8°C long term (12 months). Store in the dark.

Overview

Reducing sugars (RS) are widely present in animals, plants, microorganisms, and cultured cells. Reducing sugars in plants primarily include glucose, fructose, and maltose. Among these, glucose and fructose are not only the main substrates for respiration but also serve as substrates for the further synthesis of sucrose, starch, and cellulose.

Detection Principle: In an alkaline solution, 3,5-dinitrosalicylic acid (DNS) can be reduced by reducing sugars to produce a brown-red-colored amino compound, which has a characteristic absorption peak at 540 nm. Within a certain concentration range, the RS content is linearly correlated with the absorbance at 540 nm. The RS content in the sample can be calculated based on a standard curve.

Detection Range: 0.05 - 0.6 mg/mL

Sensitivity: 0.025 mg/mL

Applicable Samples: Plant tissues, animal tissues, cells, bacteria, serum (plasma)

component

R1501790	Component	48T	96T	Storage
R1501790A	Extraction Buffer	60 mL	120 mL	2-8°C
R1501790B	DNS Reagent	10 mL	20 mL	2-8°C. Store in the dark.
R1501790C	Standard	1EA	1EA	2-8°C

Note: Before formal testing, it is recommended to perform a preliminary test with 2-3 samples expected to have significant differences.

User-Prepared Instruments and Reagents

- Microplate reader or visible spectrophotometer (capable of measuring absorbance at 540 nm)
- 96-well plate or micro glass cuvettes, adjustable micropipettes and tips
- Centrifuge, water bath
- Deionized water
- Homogenizer (for tissue samples)

Experimental Procedure

1. Reagent Preparation

Reagent Name	Reagent Preparation	Notes
Extraction Buffer	Ready-to-use; Equilibrate to room temperature before use.	Store at 4°C. Slightly irritating. Use appropriate personal protective equipment.
DNS Reagent	Ready-to-use; Equilibrate to room temperature before use.	Store at 4°C protected from light. Slightly irritating. Use appropriate personal protective equipment.
Standard	Before use, add 1 mL of deionized water to dissolve, preparing a 10 mg/mL stock standard solution.	Can be stored at 4°C for 2 weeks.

2. Standard Curve Setup

Dilute the 10 mg/mL standard stock solution with deionized water to concentrations of 0.6, 0.5, 0.4, 0.3, 0.2, 0.1, and 0.05 mg/mL.

Tube	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

Note: The standard curve must be generated with each experiment. Diluted standard solutions are unstable and must be used within 4 hours.

3. Sample Preparation

3.1 Plant or Animal Tissue Samples

Weigh approximately 0.1 g of tissue. Add 1 mL of Extraction Buffer and homogenize in an ice bath. Transfer the homogenate to a capped centrifuge tube (to prevent evaporation during heating). Incubate in an 80°C water bath for 40 minutes, vortexing every 5 minutes. Centrifuge at 8,000 g, 25°C for 10 minutes. Collect the supernatant for assay.

3.2 Bacteria or Cells

Collect bacteria or cells into a centrifuge tube; discard the supernatant. Add 1 mL of Extraction Buffer per 5 million bacteria/cells. Sonicate in an ice bath for 5 minutes (power 20%, pulse 3s on, 10s off, repeat 30 times). Transfer to a capped centrifuge tube (to prevent evaporation during heating). Incubate in an 80°C water bath for 40 minutes, vortexing every 5 minutes. Centrifuge at 8,000 g, 25°C for 10 minutes. Collect the supernatant for assay.

3.3 Serum (Plasma) Samples

Take 0.1 mL of serum (plasma) and add 0.9 mL of Extraction Buffer; mix thoroughly. Transfer to a capped centrifuge tube (to prevent evaporation during heating). Incubate in an 80°C water bath for 40 minutes, vortexing every 5 minutes. Centrifuge at 8,000 g, 25°C for 10 minutes. Collect the supernatant for assay.

Note:

- If protein concentration measurement is required, Aladdin's BCA Protein Quantification Kit (B665595) or Ready-to-Use BCA Protein Quantification Kit (R1491648) is recommended. The Extraction Buffer contains components that denature proteins. If calculating based on protein concentration, protein needs to be re-extracted separately for measurement.

4. Assay Steps

4.1 Preheat the microplate reader or visible spectrophotometer for at least 30 minutes. Set the wavelength to 540 nm. For spectrophotometers, zero the instrument with deionized water.

4.2 Assay Procedure:

Reagent	Blank Tube (μL)	Standard Tube (μL)	Test Tube (μL)	Control Tube (μL)
Sample	0	0	175	175
Standard (various conc.)	0	175	0	0
Deionized Water	175	0	0	125
DNS Reagent	125	125	125	0

Mix well. Heat in a boiling water bath for 5 minutes (cap tightly to prevent evaporation). Remove and immediately cool to room temperature. Transfer 200 μL to a 96-well plate or micro glass cuvette. Measure the absorbance at 540 nm. Calculate $\Delta A_{\text{test}} = A_{\text{test}} - A_{\text{control}}$, $\Delta A_{\text{standard}} = A_{\text{standard}} - A_{\text{blank}}$.

Note:

- The Blank and Standard tubes only need to be set up 1-2 times.
- It is recommended to perform a preliminary test with 2-3 samples expected to have significant differences before the formal experiment. If ΔA_{test} is less than 0.04, consider increasing the sample volume appropriately. If ΔA_{test} is greater than the $\Delta A_{\text{standard}}$ of the 0.6 mg/mL standard, further dilute the sample with Extraction Buffer (multiply the result by the dilution factor) or reduce the amount of sample used for extraction.

5. Calculation of Results

Note: We provide both the derived formula and a simplified formula. They are equivalent. It is recommended to use the **simplified formula in bold** for final calculation.

5.1 Standard Curve Plotting

Plot the standard concentration (y-axis) against $\Delta A_{\text{standard}}$ (x-axis) to generate the standard curve. Substitute ΔA_{test} into the standard curve equation to calculate y (mg/mL).

5.2 Sample Reducing Sugar Content Calculation

(1) Based on Sample Weight

$$\text{Reducing Sugar } (\mu\text{g/g}) = 1000 \times y \times V_{\text{extraction}} \div W \times n = 1000 \times y / W \times n$$

(2) Based on Sample Protein Concentration

$$\text{Reducing Sugar } (\mu\text{g/mg prot}) = 1000 \times y \times V_{\text{extraction}} \div (V_{\text{extraction}} \times \text{Cpr}) \times n = 1000 \times y / \text{Cpr} \times n$$

(3) Based on Bacterial or Cell Count

$$\text{Reducing Sugar } (\mu\text{g}/10^4) = 1000 \times y \times V_{\text{extraction}} \div 500 \times n = 2 \times y \times n$$

(4) Based on Serum (Plasma) Volume

$$\text{Reducing Sugar } (\mu\text{g/mL}) = 1000 \times y \times V_{\text{extraction}} \div V_{\text{liquid}} \times n = 10000 \times y \times n$$

Parameter Definitions:

- 1000: Unit conversion factor (1 mg/mL = 1000 $\mu\text{g/mL}$)
- $V_{\text{extraction}}$: Volume of Extraction Buffer added (1 mL)
- V_{liquid} : Volume of serum (plasma) added (0.1 mL)
- Cpr: Sample protein concentration (mg/mL)
- W: Sample weight (g)
- 500: Total number of bacteria or cells (5 million)
- n: Dilution factor

6. Representative Results

Typical Standard Curve: $y = 0.2243x + 0.0545$, $R^2 = 0.9957$

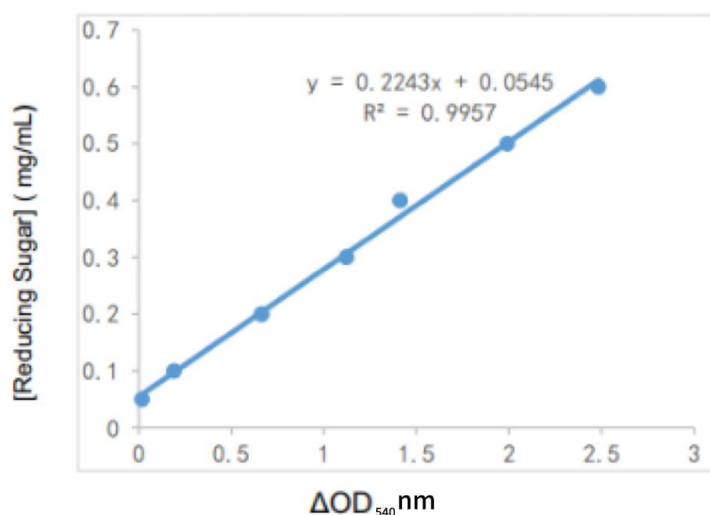


Figure 1. Typical standard curve.

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