# Reducing Sugar Content Assay Kit

**Note:** Take two or three different samples for prediction before test.
**Operation Equipment:** Spectrophotometer
**Catalog Number:** NA0851
**Size:**50T/24S

**Components:**
Reagent I: 100 mL×1.  Storage at 4℃.
Reagent II: 35 mL×1. Storage at 4℃.
Standard: powder×1, stored at 4℃. Containing 10 mg of anhydrous glucose (loss on drying < 0.2%). Add 1 mL of distilled water to dissolve it for standby before use. It can be stored for one week at 4℃, or it can be stored for a longer time with saturated benzoic acid solution.

**Product Description**
Reducing sugar is widely found in animals, plants, microorganisms and cultured cells. Reducing sugars in plants mainly include glucose, fructose and maltose, which are the most common monosaccharides and disaccharides. Glucose and fructose are not only the main substrates of respiration, but also the substrates for further synthesis of sucrose, starch and cellulose.
Heating can promote the formation of brownish red amino compound from 3,5-Dinitrosalicylic acid solution and reducing sugar in alkaline solution. This brownish red amino compound has a characteristic absorption peak at 540 nm. Within a certain concentration range, the content of reducing sugar has a linear relationship with the absorbance at 540 nm. According to the standard curve, the amount of reducing sugar in the sample can be calculated.

**Reagents and Equipment Required but Not Provided.**
Spectrophotometer, water-bath, table centrifuge, transferpettor, 1 mL glass cuvette, mortar/ homogenizer, distilled water.

**Procedure**
**I. Extraction of reducing sugar**
a. Bacteria or cell treatment:
Collect the bacteria or cells into the centrifuge tube, discard the supernatant after centrifugation. The bacteria or cells ($10^4$) : the volume(mL) of Reagent I is 500~1000: 1 (It is suggest to add 2 mL of Reagent I to 10 million of bacteria or cells), ultrasonic broke bacteria or cells (ice bath, power of 20% or 200 W, ultrasound for 3 s, interval of 10 s, repeat 30 times). Transfer to the covered centrifuge tube (to prevent water loss during heating), water bath at 80℃ for 40 minutes and during which shake for 8-10 times. Centrifuge at 8000 ×g for 10 minutes at 25℃, take the supernatant for determination.
b. Tissue:

The proportion of tissue mass (g): the volume(mL) of Reagent I is 1:5-10 (it is recommended to weigh about 0.2 g of tissue and add 2 mL of Reagent I), ice bath homogenate. Transfer to a covered centrifuge tube (to prevent water loss during heating), water bath at 80℃ for 40 minutes and during which shake for 8-10 times. Centrifuge at 8000 ×g for 10 minutes at 25℃, take the supernatant for determination.

c. Treatment of serum (slurry):

The proportion of serum (slurry) volume (mL): Reagent I volume(mL) is 1:5 ~ 10 (it is recommended to take 0.2 mL of serum (slurry) and add 1.8 mL of Reagent I), ice bath homogenate. Transfer to covered centrifuge tube (to prevent water loss during heating), water bath at 80℃ for 40 minutes and during which shake for 8-10 times. Centrifuge at 8000 ×g for 10 minutes at 25℃, take the supernatant for determination.

**II. Determination procedure:**

a. Preheat the spectrophotometer for 30 minutes, adjust the wavelength to 540 nm and adjust zero with distilled water.

b. Standard preparation: Dilute the standard with distilled water to 0.25, 0.2, 0.15, 0.1 and 0.05 mg/mL.

c. Add the following reagents successively into the EP tube:

| Reagent (μL) | Contrast Tube (C) | Test Tube (T) | Standard Tube (S) | Blank Tube (B) |
|---|---|---|---|---|
| Sample | 700 | 700 | - | - |
| Standard solution | - | - | 700 | - |
| Reagent II | - | 500 | 500 | 500 |
| Distilled water | 500 | - | - | 700 |

d. Mix, heat in boiling water bath for 5 minutes (cover tightly to prevent water loss), cool to room temperature immediately after taking out, mix well. Read the absorbance values of standard tube, contrast tube, test tube and blank tube at 540 nm. Calculate $\Delta A=A_T-A_C$.

Standard curve: According to the concentration and absorbance of the standard tube ($A_S-A_B$), establish the standard curve, x is the absorbance value, y is the concentration of the standard (mg/mL).

**III. Calculation of reducing sugar content:**

1. Calculate the content of reducing sugar in the sample according to the standard curve. Take $\Delta A(A_T-A_C)$ into x to obtain y value by calculate.

2. Calculate by Sample fresh weight:

Reducing sugar content (μmol/g fresh weight) =1000×y×V1÷W=2000×y÷W

3. Calculate by the protein concentration:

Reducing sugar content (μmol/mg prot) = (1000×y×V1)÷(V1×Cpr) =1000×y÷Cpr

4. Calculate by the number of bacteria or cells

Reducing sugar content (μmol/$10^4$ cell) = 1000×y×V1÷(500×V1) =2×y

5. Calculate by the volume of serum (plasma):

Reducing sugar content (μmol/mL) =1000×y×V2÷V3=10000×y

1000: Unit conversion coefficient, 1 mg/mL = 1000 μg/mL;

V1: Add the volume of Reagent I, 2 mL;

V2: Add the total volume of serum and Reagent I, 2 mL;

V3: Add the volume of serum (plasma), 0.2 mL;
Cpr: Sample protein concentration, mg/mL;
W: Sample fresh weight, g;
500: Total number of bacteria or cells, 5 million.

**Notes:**

1．Each test tube shall be provided with a contrast tube.

2．If the absorbance value exceeds the linear range, the sample size can be increased or diluted before determination

**References:**

[1] Lindsay H. A colorimetric estimation of reducing sugars in potatoes with 3, 5-dinitrosalicylic acid[J]. Potato Research, 1973, 16(3): 176-179.

[2] Breuil C, Saddler J N. Comparison of the 3, 5-dinitrosalicylic acid and Nelson-Somogyi methods of assaying for reducing sugars and determining cellulase activity[J]. Enzyme and microbial technology, 1985, 7(7): 327-332.

[3] Brunton N P, Gormley T R, Murray B. Use of the alditol acetate derivatisation for the analysis of reducing sugars in potato tubers[J]. Food chemistry, 2007, 104(1): 398-402.

**Related Products:**

NA0841/NA0599 Trehalose Content Assay Kit
NA0840/NA0598 Glucogen Content Assay Kit
NA0689/NA0448 Sorbitol Dehydrogenase(SDH) Activity Assay Kit
NA0869/NA0626 Plant Soluble Sugar Content Assay Kit

**Technical Specifications:**

Minimum Detection Limit: 0.0487 mg/mL
Linear Range: 0.05-0.25 mg/mL