Reducing Sugar Content Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Spectrophotometer

Catalog Number: BC0230

Size:50T/24S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Solarbio staff in time.

Reagent name	Size	Preservation Condition
Reagent I	Liquid 40 mL×1	2-8℃
Reagent II	Liquid 25 mL×1	2-8℃
Standard	Powder×1	2-8℃

Solution Preparation:

1. **Standard**: 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 two weeks at 2-8℃, 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, sonicator, 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 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

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 $\Delta A=A_T-A_C$, $\Delta A_S=A_S-A_C$. Blank tubes and standard curves only need to be done 1-2 times.

III. Calculation of reducing sugar content:

1. Standard curve:

According to the concentration and absorbance of the standard tube (A_S-A_B), establishthe standard curve, x is the absorbance value, y is the concentration of the standard (mg/mL).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/104 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;
1000: Total number of bacteria or cells, 10 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.

Recent Product Citations:

[1] Lai Y, Deng H, Fang Q, Ma L, Lei H, Guo X, Chen Y, Song C. Water-Insoluble Polysaccharide Extracted from Poria cocos Alleviates Antibiotic-Associated Diarrhea Based on Regulating the Gut Microbiota in Mice. Foods. 2023 Aug 16;12(16):3080. doi: 10.3390/foods12163080. PMID: 37628079; PMCID: PMC10453245.

[2] Xue SJ, Li XC, Huang X, Liu J, Li Y, Zhang XT, Zhang JY. Diversity investigation of cultivable yeasts associated with honeycombs and identification of a novel Rhodotorula toruloides strain with the robust concomitant production of lipid and carotenoid. Bioresour Technol. 2023 Feb;370:128573. doi: 10.1016/j.biortech.2022.128573. Epub 2023 Jan 2. PMID: 36603754.

[3] Lian T, Zhang W, Cao Q, Wang S, Dong H, Yin F. Improving production of lactic acid and volatile fatty acids from dairy cattle manure and corn straw silage: Effects of mixing ratios and temperature. Bioresour Technol. 2022 Sep;359:127449. doi: 10.1016/j.biortech.2022.127449. Epub 2022 Jun 10. PMID: 35697263.

[4] Wang Y, Liu L, Pu X, Ma C, Qu H, Wei M, Zhang K, Wu Q, Li C. Transcriptome Analysis and SNP Identification Reveal That Heterologous Overexpression of Two Uncharacterized Genes Enhances the Tolerance of Magnaporthe oryzae to Manganese Toxicity. Microbiol Spectr. 2022 Jun 29;10(3):e0260521. doi: 10.1128/spectrum.02605-21. Epub 2022 May 31. PMID: 35638819; PMCID: PMC9241697.

[5] Xiao X, Wang Q, Ma X, Lang D, Guo Z, Zhang X. Physiological Biochemistry-Combined Transcriptomic Analysis Reveals Mechanism of Bacillus cereus G2 Improved Salt-Stress Tolerance of Glycyrrhiza uralensis Fisch. Seedlings by Balancing Carbohydrate Metabolism. Front Plant Sci. 2022 Jan 4;12:712363. doi: 10.3389/fpls.2021.712363. PMID: 35058941; PMCID: PMC8764457.

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:

BC0330/BC0335	Trehalose Content Assay Kit
BC0340/BC0345	Glucogen Content Assay Kit
BC2530/BC2535	Sorbitol Dehydrogenase(SDH) Activity Assay Kit
BC0030/BC0035	Plant Soluble Sugar Content Assay Kit

Technical Specifications:

Minimum Detection Limit: 0.0487 mg/mL

Linear Range: 0.05-0.25 mg/mL