Customer service: service@abbkine.com
Technical support: support@abbkine.com
Website: https://www.abbkine.com

CheKine™ Micro Reducing Sugar (RS) Assay Kit

Cat #: KTB1360 Size: 48 T/96 T

	Micro Reducing Sugar (RS) Assay Kit		
REF	**Cat #:** KTB1360	LOT	**Lot #:** Refer to product label
	Detection range: 0.05-0.6 mg/mL		**Sensitivity:** 0.025 mg/mL
	Applicable samples: Plant Tissues, Animal Tissues, Cells, Bacteria, Serum, Plasma		
	Storage: Stored at 4℃ for 12 months, protected from light		

Assay Principle

Reduction Sugars (RS) are widely in animals, plants, microorganisms and cultured cells. The RS in plants mainly include glucose, fructose and maltose. Glucose and fructose are not only the main substrates of respiration, but also the substrate for further synthesis of sucrose, starch and cellulose. CheKine™ Micro Reduction Sugar (RS) Assay kit can detect the RS concentration from liquid samples, such as animal and plant tissues homogenate, bacteria, cells and serum (plasma). The principle of kit is that 3,5-dinitrosalicylic acid can be reduced to brown-red amino compounds by RS in alkaline solution. There is a characteristic absorption peak 540 nm. In a certain concentration range, the RS content is linearly related to the absorbance of 540 nm. According to the standard curve, the RS content in the sample can be calculated.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	50 mL	100 mL	4℃
DNS Reagent	10 mL	20 mL	4℃, protected from light
Standard	1	1	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)

Reagent Preparation

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

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

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.

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

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: It will be better to quantify the total protein with Protein Quantification Kit (BCA Assay), Cat #: KTD3001, if the content is calculated by protein concentration.

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
DNS Reagent	125	125	125	0

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 $\Delta A_{Test}=A_{Test}-A_{Blank}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

Data Analysis

Note: 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 (μg/g)=1,000×y×$V_{Extraction}$÷W×n**=1,000×y÷W×n**

(2) According to sample protein concentration

RS (μg/mg)=1,000×y×$V_{Extraction}$÷($V_{Extraction}$×Cpr)×n**=1,000×y÷Cpr×n**

(3) By the number of bacteria or cells

RS (μg/10^4)=1,000×y×$V_{Extraction}$÷500×n**=2×y×n**

(4) By the volume of serum (plasma)

RS (μg/mL)=1,000×y×$V_{Extraction}$÷V_{Sample}×n**=10,000×y×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.

Typical Data

Typical standard curve:

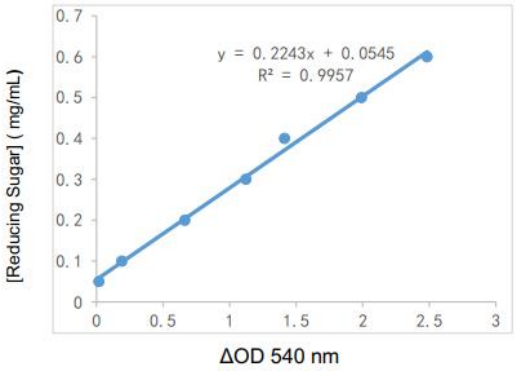

Figure 1. Standard curve for Reduction Sugar.

Recommended Products

Catalog No.	Product Name
KTB1320	CheKine™ Micro Plant Soluble Sugar Assay Kit
KTB1330	CheKine™ Micro Blood Glycogen Assay Kit
KTB1340	CheKine™ Micro Glycogen Assay Kit
KTB1350	CheKine™ Micro Total Carbohydrate Assay Kit

Disclaimer

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