Sodium Assay Kit

(Cat/No.:BC060 Size:30T/25S)

1. Composition (The kit is valid for 6 months)

Reagent composition	Specification	Storage conditions
Reagent 1	24mL bottle of liquid	4℃
Reagent 2	8mL bottle of liquid	4℃
Standard products	0.25mL liquid per bottle (See label for concentration)	4℃

Note:The reagent kit has a shelf life of 6 months and should be used within 1 month after opening.

2. Principle

The enzymatic kinetic reaction of substrate ONPG (o-nitrophenyl-β-D-thiogalactoside) catalyzed by steel-dependent β-galactosidase was monitored. Its product, o-nitrophenol, exhibits a characteristic absorption peak at 405 nm, with absorbance linearly correlated to its concentration.

$ONPG + Na^{+} \xrightarrow{\beta\text{-}galactosid\ ase} o\text{-}Nitropheno\ \ l + Lactose$ (ONPG:O-nitrophenol-β-D-galactopyranose)

3. Required Instruments and Reagents

Visible spectrophotometer and 1mL volumetric cuvette, 37℃ water bath, deionized water, protein assay reagent (for tissues, bacteria, and cells, available from our company).

4. Operating Procedures

(1) Sample pretreatment:

Liquid samples such as serum (plasma) : can be used directly (if solid matter is present, centrifuge at 4000 rpm/min for 5 min and take the supernatant for testing);

Animal (plant) tissues : Accurately weigh the tissue, add 9 times the volume of deionized water at a ratio of weight (g):volume (mL) = 1:9 , homogenize in an ice water bath, centrifuge at 4000-8000 rpm for 10 minutes, and take the supernatant of the homogenate for testing (the protein concentration of animal tissue homogenate supernatant needs to be determined; protein assay kits are available from our company; the protein concentration of plant tissue homogenate does not need to be determined) .

Bacterial/cell samples : Collect bacteria or cells into centrifuge tubes (remove the culture medium). Add 0.3-0.5 mL of deionized water for every 5 million bacteria or cells, resuspend the cells, and sonicate (ice bath, 20% power or 200W, run for 5 seconds, pause for 15 seconds, repeat 5-10 times). Centrifuge at 4000-8000 rpm for 10 minutes. Collect the supernatant for analysis (the protein concentration in the supernatant can be determined; the protein assay reagent BC016 is available from our company . If cell counting was performed earlier, protein analysis is

not required).

(2) Operation Table:

	Blank tube	Standard tube	Testing tube
Deionized water (μL)	24		
Standard (μL)		24	
Sample (μL)			24
Reagent 1 (μL)	720	720	720
Vortex mix, then bathe in a 37°C water bath for 5 minutes.			
Reagent 2 (μL)	240	240	240
Vortex mix well, keep warm at 37℃ for 1 minute and start timing. Measure the color at 405nm using a spectrophotometer at 10 seconds (recorded as A1). After colorimetric measurement, pour the liquid back into the original test tube and place it in a 37℃ water bath for reaction. Measure the color again at 405nm at 2 minutes and 10 seconds (recorded as A2). ΔA = A2 - A1.			

Note : The time interval between readings of tubes A1 and A2 must be accurately controlled, otherwise it will affect the results; for the second colorimetric test, the tube can be taken out of the 37℃ water bath 10-20 seconds in advance, poured into the cuvette, and the data should be recorded after 2 minutes and 10 seconds; at least one blank tube and one standard tube should be made for each batch of experiments.

5. Calculation Formula

1) Calculation of liquid samples such as serum (plasma):

$$Serum\ (plasma)\ sodium\ content\ (mmol/L) = \frac{\Delta A_{sample} - \Delta A_{blank}}{\Delta A_{standard} - \Delta A_{blank}} \times C_{standard} \times N$$

2) Calculation of animal tissue (or bacterial, cell) samples:

$$Sodium\ content\ (mmol/g\ protein) = \frac{\Delta A_{sample} - \Delta A_{blank}}{\Delta A_{standard} - \Delta A_{blank}} \times C_{standard} \div Cpr$$

3) Calculation of plant tissue samples:

$$Sodium\ content\ (mmol/g\ fresh\ weight) = \frac{\Delta A_{sample} - \Delta A_{blank}}{\Delta A_{standard} - \Delta A_{blank}} \times C_{standard} \div \frac{W}{V_{Total}}$$

4) Calculation of bacterial and cell samples: (Bacteria and cells can also be calculated using the formula in point 2)

$$Sodium\ content\ (mmol/10^4\ cells) = \frac{\Delta A_{sample} - \Delta A_{blank}}{\Delta A_{standard} - \Delta A_{blank}} \times C_{standard} \div \frac{Totalcellcount}{V_{Total}}$$

In the above formula, C represents the concentration of the standard (see label for concentration), in mmol/L;

N is the dilution factor of the sample before testing;

Cpr is the concentration of tissue (or bacteria, cells) protein, in g/L;

W represents the tissue weight, unit: g

V Total is the volume of homogenizing medium (deionized water) added during sample homogenization.

6. Relevant Parameters

1. Linear range: 80–180 mmol/L;
2. Reagent blank absorbance A blank ≤ 0.500;
3. Inter-batch CV ≤ 10.0%;
4. Human serum reference value: 136-146 mmol/L.

7. Precautions

1. Two samples can be taken for preliminary testing before the sample test. If the sodium concentration in the sample is too low, the amount of sample added can be increased (the amount of deionized water and standard in the blank tube and standard tube should also be increased accordingly, but the standard needs to be diluted before adding), while the amount of reagents one and two remains unchanged; if the sodium content in the sample is high, it needs to be diluted before testing.
2. It is not recommended to mix reagents from different batches;
3. Be aware of sodium ion contamination during operation ;
4. There will be no significant interference when the bilirubin is ≤0.5g/L, Hb is ≤0.5g/L, TG is ≤30g/L, and VC is ≤0.5g/L in the sample.