Niacin Kit（Ready to use）

（Product No. GVT2005 ）

GFAD[01]05.24

1. Introduction

This product is a ready-to-use kit for niacin (or niacinamide) concentration by the microbiological assay technique .developed in accordance with the standard “GB5009.89-2023”， each product box contains 3 sets of reagents.

2. Principle of the Method

Niacin (or niacinamide) an essential nutrient for the growth of *Lactiplantibacllus plantarum* (ATCC 8014). Under certain controlled conditions, *Lactiplantibacllus plantarum* suspension is inoculated into a culture medium containing the sample solution. After a period of incubation, the transmittance (or absorbance) is measured. The niacin (or niacinamide) content in the sample is then calculated based on the standard curve of folic acid content versus transmittance (or absorbance).

3. Reagents provided

Niacin Standards（Freeze-dried）	3 vials
Niacin Bacterial Ball（Freeze-dried）	3 vials
Niacin Medium Base (20 mL)	3 vials
Niacin Medium Additive (Freeze-dried)	3 vials
Sterile Water(30mL)	3 vials
Sterile 96-well Microplate individually packed	3 plates
Sealing Film	3 pieces

4. Storage condition:

Store in dark place 2-8℃ for a year

5. Materials required but not provided

5.1 Ultra-clean workstation

5.2 Constant temperature incubator, 36℃ ±1℃

5.3 Microplate reader (540 nm ~610 nm)

5.4 Autoclave

5.5 Ultrasonic oscillator

5.6 Vortex mixer

5.7 Pipette and sterile tips, 10-100 μL, 100-1000 μL, 500-5000 μL,

5.8 conical flask and volumetric flask

5.9 Pipette centrifuge tubes: 1.5 mL or 2 mL,15 mL tubes should have screw tops

5.10 Sterile syringes and 0.22 μm sterile filter

membrane

6. Assay medium preparation (aseptic procedure)

6.1 Preparation of Niacin Assay Medium: Pipette 1.0mL of the basic niacin assay medium into the additive of the Niacin Assay Medium, dissolve for 3 minutes to ensure complete mixing, and then transfer the entire mixture into 20mL of the basic Niacin Assay Medium, and mix well.

6.2 Non-inoculation of standard 0 control tube medium: 200 μL of the aforementioned medium was taken and placed in a 1.5mL sterile centrifuge tube, which serves as the medium for the non-inoculated standard 0 control tube

6.3 Inoculation of Niacin Assay Medium: take 1 vial of of niacin bacterial ball , add it to the prepared Niacin assay medium (6.1), mix well and then use.

7. Preparation of standard tubes (aseptic operation)

Accurately pipette 1.25 mL of sterile water into the Niacin Standard, dissolve and mix thoroughly. Then take 1 mL of the solution and add it to 9 mL of sterile water, mix well, and this is the niacin standard working solution. Take 10 sterile 1.5 mL(or 2mL) centrifuge tubes and prepare a series of standard solutions with varying concentrations according to Table 1.

Table 1Preparation of standard curves

Number	UN	IN	S1	S2	S3	S4	S5	S6	S7	S8
niacin content /ng	0.00	0.00	10	20	30	40	50	60	80	100
Standard solution /μL	0	0	100	200	300	400	500	600	800	1000
Sterile Water /μL	1000	1000	900	800	700	600	500	400	200	0

8. Preparation of Sample Series Tubes

8.1The sample is prepared, extracted, and diluted according to the corresponding step "12.2" outlined in GB 5009.89-2023, with the dilution range doubled compared to the national standard, resulting in a nicotinic acid content of 10ng/mL to 24ng/mL in the diluted sample extract.

8.2 The prepared sample diluent is filtered and sterilized under sterile conditions using a sterile aqueous phase filter membrane (0.22μm). and a series of sample tubes are prepared in the order specified in Table 2.

Table 2: Preparation of specimen tubes

Sample tube number	1	2	3	4
Sample solution /μL	200	400	600	800
Sterile Water /μL	800	600	400	200

9. Detection steps (aseptic operation)

9.1 Take out the sterile 96-well microplate, record the well positions, and conduct a parallel test in triplicate for each gradient of the standard solution as well as the sample diluent.Additionally, prepare one well and add 150 μL of sterile water to serve as a non-inoculated standard 0 control (UN)
9.2 Add 150 μL of prepared inoculated Niacin Bacterial Ball assay medium (6.3) to each well, and add 150 μL of uninoculated Niacin Bacterial Ball assay medium(6.2) to the standard 0 control tube
9.3 Transfer 150 μL of the standard series tube and sample series tube into the designated well.
9.4 Seal the wells on the strip with a sealing film and press the film to ensure that all wells are adequately sealed.(The edge part of the wells section should be fully sealed, with special attention paid to it.)

10. Incubation

Incubate at 36°C ± 1°C for 44-48 h in an incubator, avoiding light.

11.Measurement

11.1 Take out the plate from the incubator and press the sealing film again to ensure that all wells are adequately sealed. Shake the plate upside down repeatedly to mix the microorganisms well.
11.2 Remove the sealing film diagonally and puncture the air bubbles on the surface of each well with a needle.
11.3 Measure the absorbance value at 540 ~550nm or 610 ~630nm with a microplate reader. The culture medium in the non-inoculated standard 0 control (UN) should be clear, otherwise the assay will not be effective

12. Data analysis

Analyze the results according to 'Part 13: Expression of Analytical Results' in the national standard GB 5009.89-2023.
12.1 Standard curve: Using the niacin concentration of the standard series tubes as the abscissa and the average transmittance (or absorbance) of each standard point as the ordinate, a standard curve is plotted.
12.2 Result calculation:
The niacin content of the sample is calculated according to formula (1)

$$X = \frac{\rho \times V}{m} \times \frac{V_1}{V_2} \times f \times \frac{100}{1\,000} \quad \cdots\cdots\cdots(1)$$

X—Niacin (or nicotinamide) content in the sample diluent, ng/mL;

ρ —The total average value of the mass concentration of nicotinic acid (or nicotinamide) in the sample extract, ng/mL;

V—The fixed volume of the sample extract, mL

m—The mass or volume of the sample in grams (g) or milliliters (mL).

V_1—The constant volume in milliliters (mL) before filtration.

V_2—The volume of the filtrate in milliliters (mL) after filtration.

f — Dilution factor of sample extract;

100 — conversion coefficient

1000 — conversion coefficient

The result of the calculation retains three significant figures.

Note: For samples such as beverages for special purposes that are directly diluted to a constant volume without treatment, V1 and V2 should be removed from the formula.

For laboratory use in industry or R&D purpose. Not for drug, household or other uses.

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