Biotin Assay Kit

(Product No. VT3103)

BTN[03]08.18

1. Introduction

The test kit uses the specificity of *Lactobacillus plantarum* ATCC 8014 for biotin to determine the amount of biotin through the turbidity formed by bacterial growth in samples containing biotin.

2. Principle of the Method

The growth intensity (turbidity) of *Lactobacillus plantarum* is linearly related to the amount of biotin in the medium containing all nutrients except biotin. The medium, *Lactobacillus plantarum* and the prepared sample extracts (or standards) were added to the 96-well microplate and *Lactobacillus plantarum* will grow until biotin is depleted. A standard curve is plotted using the turbidity of the bacteria after incubation in standards against the different concentrations of the standards, and the amount of biotin in the sample is obtained by measuring the turbidity of *Lactobacillus plantarum* in the samples on the standard curve.

3. Product properties

Procedure time: operation time: 1h; incubation time: 44-48h
Range: 0.06-0.52 μg/100 g(mL)
Recovery: 80-120%
Intra-batch variation: <10%
Inter-batch variation: <10%
Storage condition: 1 year shelf life under 2-8℃ storage

4. Reagents provided

Biotin Standards	3 vials
Biotin Test Bacterial Ball	3 vials
Biotin Medium	3 vials
1X Biotin Buffer (50 mL/vial)	3 vials
100X Biotin Detection Buffer (10 mL/vial)	1 vial
Sterile Water (10 mL/vial)	3 vials
Sterile 96-well Microplate individually packed	3 plates
Sealing Film	3 pieces

5. Materials required but not provided

5.1 Ultra-clean workstation
5.2 Microplate reader (550 or 630 nm)
5.3 Constant temperature incubator, 36℃±1℃
5.4 Autoclave
5.5 Water bath, 95℃
5.6 Vortex mixer
5.7 Pipette and sterile tips, 20-200 μL, 100-1000 μL
5.8 15mL and 50mL sterile centrifuge tubes with screw cap and 1.5 mL or 2 mL sterile centrifuge tubes
5.9 Sterile syringes and 0.22 μm sterile filter membrane
5.10 Distilled water
5.11 Graduated cylinder and 300 mL conical flask

6. Sample extraction and medium dissolution

Sample Preparation Solution (20 mL/sample): Dilute 100X Biotin Detection Buffer with distilled water to get 1X Biotin Detection Working Buffer as Sample Preparation Solution (Prepare it fresh when needed).

Note: Calculate the volume of Sample Preparation Solution required based on the number of samples to be tested. Each 96-well plate can generally accommodate the test of 8 samples and it requires 160 mL of Sample Preparation Solution, so transfer 2 mL of 100X Biotin Detection Buffer into a clean triangular flask, add 198 mL of distilled water and mix well to obtain 200 mL of 1X Biotin Detection Working Buffer as Sample Preparation Solution.

7. Sample preparation of milk powder

7.1 Weigh 1 g (to the accuracy of 0. 01 g) of milk powder in a 50 mL centrifuge tube and add 20 mL of Sample Preparation Solution, **corresponding to an extraction dilution factor of 20, which is already included in the standard curve.** Mix it well and put the tube in a water bath at 95°C for 30 min, during which mixing it periodically on a vortex (at least 5 times), then quickly cool it to below 30°C in ice water.

The following operations need to be carried out in the ultra-clean workstation:

7.2 Filter the cooled extract through a 0.22 μm filter membrane into a 2 mL sterile centrifuge tube to create a sterile sample solution.

7.3 Dilute the sterile sample extract with 1X Biotin Buffer to biotin concentrations of

approximately 0.06, 0.12 and 0.24 μg/100 g (mL).

Example of sample preparation

For example, to test the biotin concentration in an infant milk powder sample labeled with 36 μg/100g(mL), weight 1 g of sample in a 50 mL centrifuge tube and add 20 mL Sample Preparation Solution in Chapter 6. Put the tube in water bath at 95℃ for 30 min for extraction, and then quickly cool it down to below 30℃. Filter the cooled extract through a 0.22 μm filter membrane to remove bacteria. Dilute the filtered sample extract 600 times, 300 times and 150 times to obtain the final concentrations of biotin approximately at 0.06 μg/100 g (mL), 0.12 μg/100 g (mL) and 0.24 μg/100 g (mL) respectively. The dilution method is listed as follows:

Sample dilution times	Dilution protocol
①10 times	900 μL **1X Biotin Buffer** +100 μL **sample extract**
②50 times	400 μL **1X Biotin Buffer** +100 μL**① solution**
③150 times	400 μL **1X Biotin Buffer** +200 μL**② solution**
④300 times	500 μL **1X Biotin Buffer** +100 μL**② solution**
⑤600 times	550 μL **1X Biotin Buffer** +50 μL**② solution**

Note: Samples should be fully mixed after each dilution step and sample extracts must be used on the same day and stored in dark place. In case of unknown samples, two biotin concentrations should be assumed and processed separately as above, with the two assumed values being adjacent orders of magnitude, following the steps above.

8. Method procedure

8.1 Biotin Standards preparation (to be carried out in an ultra-clean workstation)

Take 1 vial of lyophilized biotin standard and add 4 mL 1X Biotin Buffer to prepare a standard solution of biotin. Take 8 sterile 1.5 mL centrifuge tubes and prepare a series of standard solutions from 0.06-0.52 μg/100g(mL) according to the table below:

μg/100 g(mL)	Volume of 0.1μg/100g(mL) standard solution (μL)		Volume of 1X Biotin Buffer (μL)		Total volume (μL)
Blank: 0	0	+	500	=	500
Standard 1: 0.06	30	+	970	=	1000

Standard 2: 0.12	60	+	940	=	1000
Standard 3: 0.20	100	+	900	=	1000
Standard 4: 0.28	140	+	860	=	1000
Standard 5: 0.36	180	+	820	=	1000
Standard 6: 0.44	220	+	780	=	1000
Standard 7: 0.52	260	+	740	=	1000

Note: Standard solutions should be prepared fresh when needed and they can not be stored.

8.2 Preparation of Biotin Medium Solution

1) Pour one vial of sterile water provided in the kit into one vial of Biotin Medium, and then tighten the cap and shake it until dissolved (sterile water provided in the kit must be used).
2) Put the biotin medium solution vial in water bath at 95°C for 5 min and shake it 2-3 times periodically during this time, and then quickly cool it in ice water to below 30°C.
3) In the ultra-clean workstation, filter the Biotin Medium Solution through a sterile 0.22 μm filter membrane into a 15 mL sterile centrifuge tube. Each vial of Biotin Medium Solution is sufficient for one 96-well microplate.

8.3 and 8.4 below need to be carried out in the ultra-clean workstation:

8.3 Preparation of Biotin Test Bacterial Solution

Dissolve 1 vial of Biotin Test Bacterial Ball in the filtered Biotin Medium Solution in 8.2, and then tighten the cap and shake it until fully mixed.

8.4 Assay procedure

1) Determine the number of microwell strips required to test the desired number of samples plus the number of wells needed for standards, considering that each sample and standard need be tested in triplet. Insert the appropriate number of strips in the holder, and record the position of the wells to create a layout. Immediately reseal the unused strips in the bag together with the desiccant bag provided and store in 2-8℃.
2) Add 100 μL of **Biotin Test Bacterial Solution in 8.3** in each well.
3) Add 100 μL of Blank Standard (zero standard) to wells A1, A2 and A3; add 100 μL of each standard solution (Standard 1-7) to wells B1, B2 and B3, C1, C2 and C3 -- H1, H2 and H3 as shown below. The concentrations of Standard 1-7 are 0.06, 0.12, 0.20, 0.28, 0.36, 0.44, 0.52 μg/100g(mL).

1	2	3	4	5	6	7	8	9	10	11	12

A	Blank	Blank	Blank									
B	St1	St1	St1									
C	St2	St2	St2									
D	St3	St3	St3									
E	St4	St4	St4									
F	St5	St5	St5									
G	St6	St6	St6									
H	St7	St7	St7									

4) Add 100 µL of each prepared sample to the remaining microtiter wells.
5) Seal the wells on the strip with a sealing film and press the film to ensure that all wells are adequately sealed.

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

8.6 Measurement

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.
2) Remove the sealing film diagonally and puncture the air bubbles on the surface of each well with a needle.
3) Measure the absorbance at 550 or 630 nm. 550nm is recommended.
Note: If the absorbance cannot be measured in time after incubation, keep the plate at 2-8°C for no more than 48 h.

9. Data analysis

9.1 Determination of validity of test results:

OD values of low concentration standards < OD values of high concentration standards

9.2 Select the optimally diluted sample to calculate the results:

For each sample, which is diluted to three different levels of concentration, select the one(s) whose OD value locates at the middle of the standard curve. In case two or more are in the middle, calculate the average result.

Use the 4-Parameter calculation formula in professional ELISA statistical analysis software to calculate the concentration of pantothenic acid in the samples. (Note: when multiple the dilution factors of samples, do NOT consider the 20X dilution during extraction.)

Note: The consumables required for the experiment must be sterile; waste must be disposed of after the experiment in accordance with the relevant regulations.

Note:
For infant and young children's complementary foods samples like baby rice cereal or baby noodles, sample preparation protocol for milk powder can also be applied. However, due to the high starch content of these products, a colloidal state is easily formed during the extraction process, which results in the incapability to apply membrane filtration directly after the extraction. In this case, the sample extract can be diluted first and then filtered to remove bacteria and debris. Use the filtrate for assay.

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

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