Pantothenic Acid Assay Kit

(Product No. VT3105)

PTD[04] 02.20

1. Introduction

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

2. Principle of the Method

The growth intensity (turbidity) of *Lactobacillus plantarum is* linearly related to the amount of pantothenic acid in the medium containing all nutrients except pantothenic acid. The medium, *Lactobacillus plantarum* and the prepared sample extracts (or standards) were added to the 96-well microplate and *Lactobacillus plantarum* will grow until pantothenic acid 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 pantothenic acid 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: 10-100 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

Pantothenic Acid Standards	3 vials
Pantothenic Acid Test Bacterial Ball	3 vials
Pantothenic Acid Medium	3 vials
1X Pantothenic Acid Buffer（50 mL/vial）	3 vials
20X Pantothenic Acid Detection Buffer (30 mL/vial)	1 vial
20X Pantothenic Acid Detection Buffer (15 mL/vial)	2 vials

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 20X Pantothenic Acid Detection Buffer with distilled water to get 1X Pantothenic Acid 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 10 mL of 20X Pantothenic Acid Detection Buffer into a clean triangular flask, add 190 mL of distilled water and mix well to obtain 200 mL of 1X Pantothenic Acid Detection Working Buffer as Sample Preparation Solution.

7. Sample preparation of milk powder

7.1 Weigh 1 g (to the accuracy of 0. 001 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 Pantothenic Acid Buffer to pantothenic acid concentrations of approximately 20, 40 and 80 μg/100 g (mL).

Example of sample preparation

For example, to test the pantothenic acid concentration in an infant milk powder sample labeled with 3.6 mg/100 g(mL), weight 1 g of sample in a 50 mL centrifuge tube and add 20 mL Sample Preparation Solution in 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 180 times, 90 times and 45 times to obtain the final concentrations of pantothenic acid approximately at 20 μg/100 g (mL), 40 μg/100 g (mL) and 80 μg/100 g (mL) respectively. For operational convenience, samples can be diluted 50X, 100X and 200X respectively. The dilution method is listed as follows:

Sample dilution times	Dilution protocol
①10 times	900μL **Standard/sample Diluent**+100μL **sample extract**
②50 times	400 μL **Standard/sample Diluent** +100 μL**① solution**
③100 times	900 μL **Standard/sample Diluent** +100 μL**① solution**
④200 times	950 μL **Standard/sample Diluent** +50 μL**① 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 pantothenic acid 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 Pantothenic Acid Standards preparation (to be carried out in an ultra-clean workstation)

Take 1 vial of lyophilized Pantothenic Acid Standard and add 5 mL 1X Pantothenic Acid Buffer to prepare a standard solution of pantothenic acid. Take 8 sterile 1.5 mL centrifuge tubes and prepare a series of standard solutions from 10 to 100 μg/100 g (mL) according to the table below:

μg/100g(mL)	Volume of standard		Volume of 1X Pantothenic		Total volume

	solution (μL)		Acid Buffer (μL)		(μL)
Blank: 0	0	+	500	=	500
Standard 1: 10	50	+	950	=	1000
Standard 2: 20	100	+	900	=	1000
Standard 3: 30	150	+	850	=	1000
Standard 4: 40	200	+	800	=	1000
Standard 5: 60	300	+	700	=	1000
Standard 6: 80	400	+	600	=	1000
Standard 7: 100	500	+	500	=	1000

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

8.2 Preparation of Vitamin B_{12} Medium Solution

1) Pour one vial of sterile water provided in the kit into one vial of Pantothenic Acid Medium, and then tighten the cap and shake it until dissolved (sterile water provided in the kit must be used).

2) Put the pantothenic acid 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 Pantothenic Acid Medium Solution through a sterile 0.22 μm filter membrane into a 15 mL sterile centrifuge tube. Each vial of Pantothenic Acid 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 Pantothenic Acid Test Bacterial Solution

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

(Note: The white sphere in the Pantothenic Acid Test Bacterial Ball bottle is pantothenic acid test bacterial ball and the coloured sphere is the stabilizers. Both of them can be added together to the medium solution during the experiment. The white sphere will dissolve after shaking, while the coloured one does not. But it does not affect the subsequent experiment or the final results.)

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 **Pantothenic Acid 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 10, 20, 30, 40, 60, 80 and 100 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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