Vitamin B7 (Biotin) Test Kit

VB7 Test

Product Number: R6001

Product Unit: 1 plate, 96T

FOR Professional and Laboratory use only

Manufacturer Information

Ring Biotechnology Co., Ltd

E-mail: export@ringbio.com diego@nbgen.com Web: www.ringbio.com

Add: Building 3, Zhongtongtai TechnoPark, No. 11, Kechuang 14th St, Beijing 100176, CHINA

Tel: +86-10-56267496 Technical Support & Service: +86-13811393460

1. Introduction

The vitamin B7 (Biotin) Test Kit is a microbiological method for the quantitative determination of the total biotin (added and natural biotin) in food, animal feed and pharmaceutical products. The kit can determine 96 times.

2. Principle of the Test

The test system is an implementation of ISO standard. Biotin is extracted from the sample and the n diluted. The diluted extract and biotin assay medium are added to the wells of a microtiter plate coated with *Lactobacillus plantarum*, which relies on the presence of biotin to grow. After adding biotin as a standard or as a vitamin contained in a sample, the germ grows until the vitamin all consumed. Incubation at 37 °C in darkness for 44-48h. The growth of Lactobacillus plantarum depending on the extracted biotin is monitored as turbidity and compared to a standard series. Then measurement the result by ELISA reader at 610-630 nm (alternatively at 540-550 nm).

3. Kit Components

(1) Microtiter plate, 96wells, 1plate

(2) Sterilized water 30ml, 3 bottles

(3) Assay Medium, 3 bottles

(4) Biotin Standard, 3 bottles

The final standard series is **0, 0.08, 0.24, 0.40, 0.56, 0.72 µg/ml**

(5) Microplate cover, 2pcs

4. Required reagents and instruments (not provided)

4.1 Reagent and Solution

(1) Sodium hydroxide NaOH, 1mol/L, 0.1mol/L

NaOH Concentrate: 40g add in 100ml sterile water or deionized water.

2mol/L NaOH solution: 8g add in 100ml sterile water or deionized water.

(2) Sulfuric acid, H2SO4, 1mol/L

(3) Hydrochloric acid, HCl, 1mol/L, 0.1mol/L

(4) Amylase Z. B. (Fluka product number 86250)

(5) Sterile water or deionized water

(6) Citrate buffer (pH 4.5):

- Take 1.5g citric acid monohydrate, add into a beaker, add **50 mL sterile water** or deionized water to dissolve by stirring.
- Add **12 mL NaOH** (1mol/L) or **0.48g NaOH.** Adjust the pH to 4.5 with 0.1mol/L HCl.
- Transferred the mixed solution to a 100 mL volumetric flask with sterile or deionized water, and made up to volume with sterile water or deionized water. **The final volume will be 100mL**.

Note: this citrate buffer solution can be stored for no more than 3 days at 2-8°C.

4.2 Instrument

(1) Aseptic bench

(2) ELISA reader 610-630nm (540-550nm)

(3) Incubator with dark chamber, 37°C (98.6°F)

(4) Water bath 95°C

(5) pH meter

(6) Centrifuge > 8000 x g

(7) Sterile pipette 20-200µL; 100-1000µL

(8) Sterile centrifuge vials with screw cap 15ml and 50ml

(9) Sterile vials 1.5-2.0 ml

(10) Spiral glass pot 500ml

(11) Volumetric flask 100 and 1000ml

(12) Beaker 100ml

(13) Sterile filters polyethersulfone 0.2µm with sterile filter

5. Sample preparation

5.1 Notice

(1) To determine the added biotin in vitamin-enriched solid samples usually extraction with hot water under alkaline conditions.

(2) To determine the added biotin in liquid samples usually requires detection after sterile filtration and dilution with sterile water.

(3) To determine the total biotin in samples must be treated with enzyme.

(4) Samples should be stored in the dark at 4°C.

(5) Standards and samples should be in **triplicate**.

(6) Unknown Samples extract should be diluted twice.

(7) The sample extract should be prepared freshly before analysis and should be stored in the dark.

5.2 Sample extraction

(1) Add 1 g of homogenized sample to 40 mL of sterile water or deionized water or extraction solution at a dilution factor of 40. The dilution factor is directly included in the standard curve.

(2) For samples with low biotin concentration, the sample volume should be increased by 5g (mL) (calculation should be taken into account)

The following samples must **be sterile filtered** or **aseptically extracted**:

1) Samples not heated at the time of extraction, such as juice or healthy drinks (excluding samples heated at 95°C in a water bath for 30 min).
2) Contains Chinese medicine and seasoning samples as well as honey and tea.
3) Vitamin mixtures, premixes or tablets (samples with higher biotin content) (except samples heated for 30 min in a 95°C water bath).
4) Darker samples with lower vitamin levels (filter step to remove color).
5) If the sample contains solid particles or the sample haze affects filtration, it should be sterile filtration before centrifuge for 5 min (> 8000 x g).

Note: The sample do not need sterile filtration if the sample heated at 95°C for 30 min.

Dilution must be carried out with the sterile water provided in the kit. (The medium must be

filtered)

5.3 Dilution (IMPORTANT)

(1) Dilution factor

Example: If a test sample with concentration of 125μg / 100g. Divide this concentration by the concentration of standard 2 to obtain the dilution factor.

Dilution factor = 80μg/0.24μg = 333

So the dilution factor should be 300, sample should be dilute at 1:300.

Dilution step

a: 1:10 (100μl sample extraction + 900μl sterile water from the test kit)

b: 1:10 (100μl Buffer a + 900μl sterile water from the test kit)

c: 1:3 (200μl Buffer b + 400μl sterile water from the test kit)

5.4 Sample preparation Protocols

5.4.1 Liquid sample (multivitamin-containing juices and healthy drinks)

Add 1 mL sample to a 50 mL sterile centrifuge vial, fill up to 40 mL with sterile water or deionized water, mix, sterile filter (or heat the sample in a 95°C water bath for 30 min, chill down quickly to below 30°C). Then transfer some of the extract to 1.5 or 2.0 mL sterile reaction vial, depending on the concentration range, further dilutions with sterile water are necessary.

5.4.2 Pectin and candy sample

(1) Weight 15-20g pectin sugar or candy, into a 50 mL sterile centrifuge vial, fill up to 40 mL with sterile water or deionized water, dissolve the sample in 95°C water bath, chill down quickly to below 30°C.

(2) The extract solution was quantitatively transferred to a 100 mL volumetric flask with sterile or deionized water. Transfer the extraction solution containing approximately **1g sample** into a 50 mL sterile centrifuge vial, fill up to 40 mL with sterile water or deionized water, mix, sterile filter (or heat the sample in 95°C water bath for 30 min, chill down quickly to below 30°C). Then transfer some of the extract to 1.5 or 2.0 mL sterile reaction vial, depending on the concentration range, further dilutions with sterile water are necessary.

For example: If the sample is 17g pectin sugar, the solution transferred to the sterile centrifuge vial (extract solution containing 1g sample) is : 100mL/17g = 5.88mL/g.

5.4.3 Capsules, Pills, and Vitamin Mixtures

Before testing:

- Determine the weight of each capsule or pill (weigh 5 capsules or pills and average them), then pulverize the pills in a mortar or mixer (capsules can be extracted directly after cutting).
- The content of biotin in capsules, pills, and vitamins is usually high. If the dilution factor (biotin content in 100 g sample divided by biotin content in standard 2) is greater than 10,000, pre-extraction is recommended before sample preparation. (1000ml sterile water or deionized water).

Sample preparation and pre-extraction with a dilution factor of 20000

(1) Weigh 1g pill, vitamin mixture or cut capsule, into a 500mL spiral glass pot, fill up to 400 mL

with sterile water or deionized water, mix. Adjust pH to 8.0±0.2 with HCl or NaOH,

(2) Heat the sample in 95°C water bath for 30 min, mix at least 5 times during the period, chill down quickly to below 30°C. The extract solution was quantitatively transferred to a 1000 mL volumetric flask with sterile or deionized water.

(3) Transfer 1mL the extract to a 50 mL sterile centrifuge vial, fill up to 40 mL with sterile water or deionized water, mix, sterile filter (or heat the sample in 95°C water bath for 30 min, chill down quickly to below 30°C).

Then use 1:20 dilution of sterile water in 1.5 or 2.0 mL sterile reaction vial.

Note: Pre-dilution factor of 20,000 should be considered when calculating the results, and 1mL to 40mL in the dilution step is included in the standard curve.

5.4.4 Cereals, baby food, bread, flour and dairy products

(1) Add 1 g of homogenized sample, into a 50 mL sterile centrifuge vial, fill up to 40 mL with sterile water or deionized water, mix, heat the sample in a 95°C water bath for 30 min, mix at least 5 times during the period, chill down quickly to below 30°C.

(2)The sample was further filtered using a 0.22 micron filter into 1.5 or 2.0 mL sterile reaction vial. According to the concentration range of biotin, the filtrate was further diluted with sterile water. Make sure the final diluted samples is within the standard solution range.

5.4.5 Determination of total biotin in liquid or solid samples

Note: the sample must be extracted with the enzyme.

(1) Weight 1g(mL) sample, into a 50 mL sterile centrifuge vial, fill up to 20 mL with sterile water or deionized water, mix, adjust pH to 4.5 with HCl.

Note: you can also use citrate buffer pH4.5 directly instead of sterile water for sample extraction, add 1g sample to 20mL pH4.5 citrate buffer, mix, without pH adjustment

(2) Add 300mg **amylase**, mix, incubation at 37 ° C in the dark for 1h (incubator or water bath), intermittent oscillations during the period. Fill up to 40 mL with sterile water or deionized water, mix, heat the sample in 95°C water bath for 30 min, mix at least 5 times during the period, chill down quickly to below 30°C.

(3) Centrifuge at more than 8000xg for 5 min. According to the concentration range of biotin, the supernatant was further diluted with sterile water in a 1.5 or 2.0 mL sterile reaction vial.

5.4.5 Determination of total biotin in yeast and yeast products

(1)Add 1 g of homogenized sample, into a 50 mL sterile centrifuge vial, fill up to 30 mL with 1 M H_2SO_4, mix, heat the sample in 95°C water bath for 30 min, mix at least 5 times during the period, chill down quickly to below 30°C.

(2) Adjust the pH to 6.0-7.0 with concentrated NaOH and 2mol/L NaOH solution.

(3) Fill up to 40 mL with sterile water or deionized water, mix, sterile filter (heat the sample in a 95°C water bath for 30 min, chill down quickly to below 30°C, centrifugal).

(4) According to the concentration range of biotin, the supernatant was further diluted with sterile water in a 1.5 or 2.0 mL sterile reaction vial.

6. Assay Protocol

6.1 Preparing biotin standard solutions

(1) Open the sterile water bottle, pill up the blue lid through the edge of the glass and remove the whole bottle cap. Open the biotin standard bottle, place the inside of the bottle cap upward.

(2) Add sterile water into the standard bottle and close the cap. Shake and dissolve the standards fully and get the standard concentrate. The volume of sterile water added is specified on be

(3)Take 6 sterile vials (1.5-2.0ml), dilute the standard concentrate according to the following table. Standards have to be prepared freshly before the test.

Standard curve in µg/100g(ml)	**Sterile water in µL**		**Standard concentrate in µl**		**Total volume in µl**
Blank: 0	900	+	0	=	900
Standard 1: 0.08	900	+	100	=	1000
Standard 2: 0.24	350	+	150	=	500
Standard 3: 0.40	250	+	250	=	500
Standard 4: 0.56	150	+	350	=	500
Standard 5: 0.72	50	+	450	=	500

6.2 Preparing biotin assay-medium

(1) Open the bottle and discard the desiccant with tweezers.

(2) Add 10ml sterile water from the test kit to the biotin assay-medium, Close the assay-medium bottle and shake well.

(3) Heat the bottle in a water bath to 95oC for 5 min while shaking at least twice. Always make sure that the bottle is tightly closed, chill down quickly to below 30°C.

(4) Filter the medium through a 0.2µm filter into a sterile 15 ml centrifuge vial.

6.3 Assay procedure

Note: The sample added to the microplate must be a sterile sample (Use the sterile water provided in the kit for dilution).

1) Remove the required number of microwell strips and place the remaining microwell strips in a desiccant-containing aluminum foil bag and store at 2-8°C.
2) Add 150 µl biotin assay medium and 150 µl standard or sample into the wells (rinse tip with standard or sample solution), Cover the strips/cavities with adhesive foil. Pull off the protective layer of the foil, place the foil flat onto the strips, smoothing it down by hand (or with a spatula), press the foil firmly onto the strips.

Note: make sure the cavities are sealed airtight by smoothing down the foil over the cavities, take special care with the wells around the edges.

3) Incubate at 37°C in the dark for 44-48 hours in an incubator.
4) Press down the adhesive foil once more, place the microtiter plate on a table and dissolve

the microorganisms thoroughly by shaking the plate on the surface of the desk.

5) Carefully pull off the adhesive foil: Fix the micro-well strip on the microwell holder with one hand, and pull the adhesive foil off the diagonally opposite corner from the upper right side with the other hand.
6) Destroy any bubbles on the surface of liquid in the wells by means of a pipette tip.
7) Measure the turbidity with an ELISA reader at 610 - 630 nm (alternatively at 540 - 550 nm).

Note: After incubation, the microtiter plate can be stored for max 48 hours in the refrigerator, thereafter the turbidity should be measured. To avoid any time losses due to weekends or bank holidays, the microtiter plate can be evaluated after 60 hours. It is recommended to use a timer to turn off the incubator after 44-48 hours.

7. Result determination

Recommend use a four points software (4 parameter logistic curve, 4PL) to calculate the results. The test results are valid if the blank OD value is below to the OD value of standard 1, and the OD value of standard 5 is greater than 0.6.

$$\text{Biotin (µg/100 ml or µg/100 g)} = \frac{\text{Concentrate read from standard curve x } \mathbf{\text{dilution factor}}}{\text{Amount of sample in ml (g)}}$$

Note: The default sample dilution factor of 40 is already included in the standard curve.
The dilution factor in the formula is the dilution factor of the sample extract.

For example:

Sample quality; 1 g

Sample dilution: 1:40 (without consider)

Sample extract dilution: 1:300 (must consider)

Reading from the standard curve: 0.24µg/100g (ml)

The actual concentration in the sample is: 0.24×300/1=72 µg/100g (ml)

Note: If biotin concentration in the sample is unknown, the sample extract should be repeated twice (the deviation should be less than 10%). If the biotin content in the high dilution factor sample solution is higher than the low dilution factor, it may indicate that there are inhibitory factors such as heavy metals and antibiotics.

8. Kit Performance

Time requirement:	Test setup in approx. 60 min
	Result calculation in 2 min
Incubation:	44-48hours in the dark at 37°C (98.6°F)
Standard Range:	0.08-0.72µg/100g (ml)
Sensitivity:	0.08µg/100g (ml)
Recovery:	90-105%
Repeatability:	C.V. < 10 %

9. Notices

1) Kits should be stored at 2-8°C. Expired kits and reagent can not be used.

For research use only. Not for use in diagnostic or therapeutic procedures.

2) The sample extract or diluent added to the microplate must be sterile, and the sample must be diluted with sterile water provided in the box.
3) Other consumables needed in the experiment must also be sterile.
4) The assay-medium could evoke irritations of mucosa, eyes and skin.
5) After running the test, the microwell strips used must be disposed of according to regulations (e.g. autoclaved).

10. Storage and expiration

The kit is valid for 12 months when stored at 2-8°C. Production and expiry information are printed on the package.