

**VH(Biotin) ELISA Kit**

Catalog No: E-FS-E138

96T

**Version Number:** V1.0  
**Replace version:** /  
**Revision Date:** 2026.05.08

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help.

Toll-free: 1-888-852-8623 Tel: 1-832-243-6086 Fax: 1-832-243-6017

Email: [techsupport@elabscience.com](mailto:techsupport@elabscience.com)

Website: [www.elabscience.com](http://www.elabscience.com)

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

## Test principle

Add the biotin solution extracted from the sample or the biotin standard solution, together with biotin-free biotin assay medium, into microwells pre-embedded with *Lactobacillus plantarum* (ATCC 8014). Incubate at 37°C in the dark for 44 - 48 h. During incubation, the bacteria grow continuously until biotin is completely depleted. There is a corresponding relationship between the growth turbidity of *Lactobacillus plantarum* and biotin content, measure the absorbance at 620 nm using a microplate reader, fit the standard working curve, and calculate the biotin content in the test sample.

## Technical indicator

**Reaction mode** (Incubation time and temperature): 37°C; 44 - 48 h.

**Limit of Quantification (LOQ):** 0.08 µg/100 g(mL).

**Sample recovery rate:** 100% ± 20%.

## Kits components

Item	Specifications
ELISA Microtiter Plate	96 wells
Biotin Assay Medium	3 vials (solid)
Biotin Standard	3 vials (solid)
Sterile Water	3 bottles (30 mL per bottle)
Adhesive Foil	2 pieces
Manual	1 copy

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution.

## Other materials required but not supplied

**Instrument:** Electronic balance, precision: 0.001 g; Laminar flow cabinet; Microplate reader, 620 nm or 630 nm; Vortex mixer; Constant temperature incubator, 37 °C; Water bath, 95 °C; Centrifuge, rotational speed ≥ 8000 rpm; pH meter, precision ±0.01.

**Micropipette:** Single channel (20-200 µL, 100-1000 µL), Multichannel (30-300 µL); Sterile pipette tips, 100–1000 µL, 20–200 µL; Sterile centrifuge tubes, 1.5 mL, 2.0 mL and 15 mL; Clean centrifuge tubes, 50 mL; Sterile syringes and 0.22 µm sterile filters; Graduated cylinder, 100 mL.

**Reagents:** Ultrapure water / Deionized water.

---

## Notes

1. The assay medium may irritate the eyes, skin and mucous membranes; handle with caution.
2. All consumables required in the experiment must be **sterile**. Preparation of standards, **sterile** filtration of medium, and **sterile** filtration or dilution of sample extract must be performed in a **sterile** environment.
3. Standard solutions and assay medium shall be freshly prepared for immediate use. Prepare three parallel wells for both standard solutions and sample solutions.
4. Follow the aspirate twice and dispense once principle during sample loading. Ensure vertical pipetting; do not let the pipette tip touch the inner wall of the microwell or the liquid inside. Replace the pipette tip for each liquid type.
5. FOR RESEARCH USE ONLY. ELISA Microtiter plate should be covered by plate sealer. Avoid the kit to strong light.
6. **Each reagent is optimized for use in the E-FS-E138. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-FS-E138 with different lot numbers.**
7. All reagents involved in this product are safe and reliable, and contain no carcinogenic, highly toxic, flammable, explosive or strongly corrosive substances.
8. Wear necessary lab coat, mask and gloves throughout the experiment. Solid and liquid waste generated after use of this product shall be disposed of in accordance with local regulatory requirements to avoid environmental contamination.
9. As the OD values of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique, washing technique or temperature effects), the operator should establish a standard curve for each test.
10. Even the same operator might get different results in two separate experiments. In order to get reproducible results, the operation of every step in the assay should be controlled.
11. **For mentioned sample fast and efficient extraction methods are included in the kit description. Please consult technical support for the applicability if other sample need to be tested.**
12. The kit is used for rapid screening of actual samples. If the test result is positive, the instrument method such as HPLC, LC/MS, etc. can be used for quantitative confirmation.

## Storage and expiry date

Store the kit at 2-8°C. Do not freeze any test kit components.

Return any unused microwells to their original foil bag and reseal them together with the desiccant provided and further store at 2-8°C. After opening, the kit is stable for up to 1 month.

**Expiry date:** expiration date is on the packing box.

## Experimental preparation

Restore all reagents and samples to room temperature before use.

Open the microplate reader in advance, preheat the instrument, and set the testing parameters.

### 1. Sample pretreatment notice:

Experimental apparatus should be clean, and the pipette should be disposable to avoid cross-contamination during the experiment.

### 2. Solution preparation

*Please prepare solution according to the number of samples. Don't use up all components in the kit at once!*

#### Solution 1: Standard Solution

(1) Open the blue cap of the **Sterile Water** bottle, pull it vertically upward along the glass edge until the entire cap is removed and discarded. Uncap the **Biotin Standard** vial and place the cap with the inner side facing upward.

(2) Add sterile water to the standard vial as required on the label, close the cap, and vortex thoroughly to fully dissolve the standard. The resulting solution is the **standard stock solution**.

(3) Take 6 sterile centrifuge tubes (1.5/2.0 mL), and dilute the biotin standard stock solution according to the table below :

Standard Curve μg/100 g (mL)	Sterile Water (μL)	Standard Stock Solution (μL)	Total Volume (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

*Note: Standard solutions shall be freshly prepared and used immediately; storage is not allowed. The dilution factor of the sample extract (40-fold) has already been included in the standard curve.*

#### Solution 2: Assay Medium

(1) Open the cap of the **Biotin Assay Medium** bottle, use tweezers to take out the desiccant and discard it.

(2) Pipette 10 mL of **Sterile Water** from the kit into the biotin assay medium vial, cap tightly and mix well.

(3) Incubate in a water bath at 95 °C for 5 min, vortex at least twice during the period and ensure the cap is tightly closed. Then cool rapidly to below 30°C.

(4) Filter the cooled medium into a 15 mL sterile centrifuge tube with a 0.22 μm sterile filter.

### 3. Sample pretreatment procedure

#### 3.1 Pretreatment of formula milk powder sample:

- (1) Weigh  $1 \pm 0.05$  g of sample into a 50 mL clean centrifuge tube, add 30 mL of ultrapure water or deionized water with a graduated cylinder and shake well. Adjust the pH to  $8.00 \pm 0.20$  with HCl or NaOH, then add ultrapure water or deionized water up to 40 mL.
- (2) Extract in a water bath at  $95^\circ\text{C}$  for 30 min, shake at least 5 times during the extraction and ensure the cap is tightly sealed.
- (3) Cool rapidly to below  $30^\circ\text{C}$ , then centrifuge at 8000 rpm for 5 min.
- (4) Further dilute the supernatant with sterile water provided in the kit according to the biotin concentration range, so that the biotin concentration in the extract falls within the middle range of the standard curve (approximately equal to the concentration of Standard 2:  $0.24 \mu\text{g}/100 \text{ g}$ ), to obtain the sample test solution.

*Example: For milk powder with a labeled biotin concentration of  $15 \mu\text{g}/100 \text{ g}$ , the dilution factor of the extract is calculated as:  $15/0.24 = 62.5$ -fold, so the approximate dilution factor is 60-fold. The subsequent dilution steps are as follows:*

- a. Mix  $100 \mu\text{L}$  sample extract with  $900 \mu\text{L}$  sterile water from the kit to prepare Solution a.*
- b. Mix  $100 \mu\text{L}$  Solution a with  $500 \mu\text{L}$  sterile water from the kit to obtain the sample test solution.*

### Assay procedure

Take the required number of microplate strips and fix them on the microplate holder. Immediately put the remaining unused strips back into the original aluminum foil bag, reseal it together with the desiccant, and store at  $2-8^\circ\text{C}$ .

1. Pipette  $150 \mu\text{L}$  of medium,  $150 \mu\text{L}$  of standard solution or  $150 \mu\text{L}$  of sample test solution into the corresponding microwells in sequence.
2. Remove the protective layer from the **Adhesive Foil**, place the adhesive foil flat on the microplate strips and press firmly by hand to ensure all microwells are fully sealed. Shake the microplate to thoroughly mix the solution in each well.
3. Incubate at  $37^\circ\text{C}$  in the dark for 44 - 48 h.
4. Press the adhesive foil tightly again. Invert the microplate on the desktop and shake gently in a smooth motion, then flip the microplate back to its original position and place it on the desktop. Carefully peel off the adhesive foil diagonally starting from the upper right corner; meanwhile, press the microplate plate with the other hand to prevent the microplate strips from being lifted due to excessive adhesion during peeling.
5. Use a pipette tip or sharp needle to break all foam on the liquid surface in each microwell. Read the absorbance with a microplate reader at 620 nm or 630 nm.

---

**Result analysis**

1. It is recommended to calculate the results using the 4-parameter method.
2. The test result is considered valid when the OD value of the blank < Standard 1, and the OD value of Standard 5 > 0.6.
3. The sample dilution factor of 40 is already included in the standard curve and does not need to be considered. Only the further dilution factor of the extract and the sample weight should be taken into account in the formula below.

$$X=(c\times f)/m$$

Note:

- X — Biotin concentration in the sample,  $\mu\text{g}/100 \text{ g}(\text{mL})$ ;
- c — Concentration read from the standard curve,  $\mu\text{g}/100 \text{ g}(\text{mL})$ ;
- f — Dilution factor;
- m — Sample weight, g

*Example:*

- (1) *Sample weight: 1 g;*
- (2) *Dilution factor of sample extraction: 40-fold (not required for calculation);*
- (3) *Further dilution factor of sample extract: 60-fold (must be included for calculation);*
- (4) *Concentration value read from the standard curve: 0.24  $\mu\text{g}/100 \text{ g}(\text{mL})$ .*

*Therefore, the actual concentration in the sample calculated according to the above formula is:*

$$0.24 \times 60 / 1 = 14.4 \mu\text{g}/100 \text{ g}.$$