

# Vitamin B2 content Assay kit

**Note:** Take two or three different samples for prediction before test.

**Operation Equipment:** High performance liquid chromatography

**Catalog Number:** BC4854

**Sizes:** 50T/48S

## Product Description:

Vitamin B2, also known as riboflavin, is an essential vitamin for body health and normal growth, widely found in yeast, liver, kidney, egg yolk, milk and soybeans. The coenzymes formed by vitamin B2 are widely involved in various REDOX reactions in the body, which can promote the metabolism of sugars, fats and proteins, and play an important role in maintaining the normal function of skin, mucous membranes and vision.

Vitamin B2 has fluorescence effect under certain conditions of light excitation, and its content can be determined by HPLC fluorescence detector. Fluorescence detectors are often used for trace analysis because of their high sensitivity.

## Reagents and Equipment Required but Not Provided:

High-efficiency liquid chromatograph (C18 column (4.6×250 mm), Fluorescence Detector (FLD), desktop centrifuge, adjustable pipette, mortar/ homogenizer, EP tube (1.5 mL), syringe filters (water), syringe, suction filter, filter membrane (organic, water), brown injection bottle, carbinol (chromatographically pure), ultrapure water, Potassium hydroxide.

## Product Composition:

**Extract solution:** 30 mL×1. Storage at 2-8°C. This extract contains insoluble matter and should be shaken well before use.

**Reagent I:** 5 mL×1. Storage at 2-8°C.

**Reagent II:** 1.5 mL×1. Storage at 2-8°C.

**Reagent III:** Powder×2. Storage at 2-8°C.

**Standard:** Powder×1. Store at 2-8°C. Before use, 1 mL 0.05mol/L Potassium hydroxide was added to prepare 5 mg/mL vitamin B2 standard solution, which was sealed and stored at 2-8°C, away from direct sunlight.

## Preparations before the experiment:

1. Dissolve 1 bottle of Reagent III into 1000 mL of ultra-pure water, then add 0.55 mL of Reagent II and mix well to obtain mobile phase A.
2. Filter 1000 mL of prepared mobile phase A and methanol (chromatographically pure) with filter membrane. (The prepared mobile phase A was filtered by 0.22μm aqueous filter membrane, and methanol was filtered by 0.45μm organic filter membrane).
3. Ultrasound the filtered mobile phase for 20 min to remove bubbles.
4. Preparation of standard products: 5 mg/mL Vitamin B2 standard solution is diluted with distilled water into 20000ng/mL、4000 ng/mL、800 ng/mL、160 ng/mL、32 ng/mL、6.4 ng/mL

## Vitamin B2 standard

solution. (The standard concentration is for reference only and can be adjusted according to the actual sample concentration). Store (sealed) at 4°C away from light, filter into brown sample bottle with water needle filter before test, to be tested.

### Procedure

#### I. Vitamin B2 extraction:

1. Tissue: According to the mass (g): Extract solution volume (mL) 1:5~10 ratio, it is recommended to weigh 0.1g sample (Fresh sample: chopped; Drying sample: Grinding and sifting) and add 0.6 mL Extract solution to fully homogenize), seal, mix evenly, and soak in a water bath at 60°C for 30 min. Cool to room temperature, add 0.1 mL of Reagent I, 0.3 mL distilled water, mix well, let stand for 2 min. Centrifuge at 10000 rpm for 10 min, take the supernatant (if there is still turbidity, it can be centrifuged again), filter it into the brown sample bottle using a water-based needle filter before the test, and then filter it again to be tested (if the color of the supernatant is too dark or the concentration is too high, it can be diluted and filtered again to be tested).

2. Cells: According to the number of cells ( $10^4$ ): the Extract solution volume (mL) is 10-50 million :1 ratio, it is recommended to take 50 million cells, add 0.6 mL of the Extraction solution, ultrasonic crushing cells (power 200W, ultrasonic 3s, intermittent 9s, repeat 30 times, total time 6 min), seal and mix well. Soak in a water bath at 60°C for 30 min. Cool to room temperature, add 0.1 mL of Reagent I, 0.3 mL of distilled water, mix well, leave for 2 min. Centrifuge at 10000 rpm for 10 min, take the supernatant (if there is still turbidity, it can be centrifuged again), and filter it into the brown sample bottle using a water-based needle filter before testing.

3. Serum: According to the serum volume (mL) : Extract solution volume (mL) 1~5:1 ratio, it is recommended to take 0.5 mL of serum, add 0.1 mL of Extract solution, seal and mix, and soak in a water bath at 60°C for 30 min. Cool to room temperature, add 0.1 mL of Reagent I, 0.3 mL of distilled water, mix well, leave for 2 min. Centrifuge at 10000 rpm for 10 min, take the supernatant (if there is still turbidity, it can be centrifuged again), and filter it into the brown sample bottle using a water-based needle filter before testing.

#### II. Determination procedure:

1. Turn on the computer, turn on the switch buttons of each module of the HPLC, install the chromatographic column, open the software, and set the injection volume in the method group to 10  $\mu$ L, column temperature: 30°C, flow rate 1 mL/min, Fluorescence detector: Ex=462 nm, Em=522 nm. The sampling time of a single sample is 25 minutes, and the preservation method group is set.

2. Use the corresponding mobile phase to clean the column, balance the column with mobile phase A, and start adding samples after the baseline is stable.

3. Test the standard solution to be measured, the sample size is 10  $\mu$ L, Vitamin B2 can be separated within 25 min, and the retention time of Vitamin B2 is about 22.8 min (the retention time is different with the system, column, mobile phase pH, temperature, etc., and is only for

reference).

4. Test the sample solution to be measured, the injection volume is 10  $\mu\text{L}$ , and test the peak area of Vitamin B2 at the corresponding retention time.
5. Complete sequence sampling table: (including the cleaning and rebalancing process of the column after the determination of a single sample is completed)

Time (t)	Carbinol (%)	Mobile phase A (%)
0 min	0	100
1 min	0	100
1.1 min	3	97
8 min	3	97
8.1 min	15	85
23 min	40	60
25 min	40	60
25.1 min	60	40
35 min	60	40
35.1 min	0	100
45 min	0	100

### III. Calculations:

The standard curve  $y=kx+b$  was drawn with the standard concentration ( $\mu\text{g}/\text{mL}$ ) as the horizontal coordinate  $x$  and the peak area as the vertical coordinate  $y$ . The peak area of the sample was substituted into the standard curve to calculate the concentration  $x$  ( $\mu\text{g}/\text{mL}$ ) of Vitamin B2 in the Extraction solution.

#### 1. Tissue sample:

$$\text{Vitamin B2 content } (\mu\text{g}/\text{mL}) = x \times V_E \div W \times F = x \div W \times F$$

$V$  extraction: Add the total volume of Extraction solution, 1 mL (0.6mL Extraction solution +0.1mL Reagent I+0.3mL distilled water);  $W$ : Sample quality, g;  $F$ : dilution ratio, the sample tested after dilution, the calculation needs to be multiplied by the corresponding dilution ratio, 1000: Unit conversion coefficient,  $1\mu\text{g}=1000\text{ng}$ .

#### 2. Cell sample:

$$\text{Vitamin B2 content } (\mu\text{g} / 10^4\text{cell}) = x \times V_E \div N \times F = x \div N \times F$$

$V$  extraction: Add the total volume of Extraction solution, 1mL (0.6mL Extraction solution +0.1mL Reagent I+0.3mL distilled water);  $N$ : Cell number,  $10^4$ ;  $F$ : dilution ratio, the sample tested after dilution, the calculation needs to be multiplied by the corresponding dilution ratio.

#### 3. Serum samples:

$$\text{Vitamin B2 content } (\mu\text{g} / \text{mL}) = x \times V_E \div V_S \times F = 2x \times F$$

$V$  extraction: Add the total volume of Extraction solution, 1mL (0.6mL Extraction solution

+0.1mL Reagent I+0.3mL distilled water);  $V_s$ : Add sample volume, 0.5mL;  $F$ : dilution ratio, the sample tested after dilution, the calculation needs to be multiplied by the corresponding dilution ratio, 1000: Unit conversion coefficient,  $1\mu\text{g}=1000\text{ng}$ .

**Note:**

## Precautions:

1. The extraction solution of this kit contains insoluble matter, which needs to be shaken well before use.
2. After the test is completed, it is necessary to flush the column with a high concentration of ultra-pure water phase (about 20-30 column volumes) to prevent blocking the column, and then flush the column with a high concentration of organic phase, and finally flush according to the type of column to prevent damage to the column.
3. The dilution times of the standard product should be determined according to the concentration of vitamin B2 in the sample, and the peak area of vitamin B2 in the sample must be within the peak area of the standard solution of different concentrations, and the dilution times of the standard product is only a reference. If the concentration of vitamin B2 in the sample is too high, it is recommended to dilute it with distilled water and then test.
4. If the sample size is too large, it is recommended to test the standard solution once a day (one concentration of the standard solution can be) to determine the corresponding retention time, and the solution to be tested must be placed at room temperature before the test.
5. In order to exclude the influence of gradient elution baseline drift, a blank detection can be performed.