Competitive enzyme immunoassay kit for quantitative analysis of Vitamin B_{12}

Catalog Number: EL2013-01

For Research Use Only. Not for use in Diagnostic Procedures

1. Background

Vitamin B_{12}, also called cobalamin, is a water-soluble vitamin that is involved in the metabolism of every cell of the human body: it is a cofactor in DNA synthesis, and in both fatty acid and amino acid metabolism. It is particularly important in the normal functioning of the nervous system via its role in the synthesis of myelin, and in the maturation of developing red blood cells in the bone marrow.

2. Test Principle

This ELISA kit is designed to detect Vitamin B12 based on "indirect-competitive" enzyme immunoassay. The microtiter wells are coated with BSA-linked metronidazole antigen. Vitamin B12 in the sample competes with the precoated antigen for binding to the limited number of antibody. After the addition of enzyme conjugate and TMB substrate, the signal is measured with an ELISA photometer. The absorption is inversely proportional to the Vitamin B12 concentration in the sample, compared with the standard curve, metronidazole residue in the sample can be calculated.

3. Applications

This kit can be used in quantitative and qualitative analysis of Vitamin B_{12} in grain (maize, soybean, millet, rice), milk powder, fresh milk, milk products.

4. Cross Reactions

Vitamin B_{12} 100%

5. Components Provided in This Kit

Component Name	Volumes	Storage	Cap Color
Microtiter Plate with 96 wells coated with antigen	1 each	15–25°C	n/a
Standard Solutions: 0ppb, 0.2ppb, 0.6ppb, 1.8ppb, 5.4ppb	5 X 1 mL	15–25°C	n/a
Spiking Standard Solution (1ppm)	1 mL	2-8°C	n/a
Enzyme Conjugate	12 mL	2-8°C	Transparent Cap
Antibody Solution	7 mL	2-8°C	Transparent Cap
Substrate Solution A	7 mL	2-8°C	White Cap
Substrate Solution B	7 mL	2-8°C	Red Cap
Stop Solution	7 mL	2-8°C	Yellow Cap
20X Concentrated Wash Solution	40 mL	2-8°C	Transparent Cap
Extraction solution	50 mL	2-8°C	Transparent Cap

*Storage period: 12 months

6. Equipment and Reagents Needed (not provided)

6.1 Equipment

- ELISA reader (450nm/630nm)
- Rotary evaporator / N_2 drying instrument
- Shaker
- Vortex mixer
- Centrifuge
- Analytical balance (inductance: 0.01g)
- Graduated pipette: 10ml
- Rubber pipette bulb
- Volumetric flask: 100ml, 500ml;
- Glass test tube: 10 ml;
- Polystyrene centrifuge tube: 50ml, 2ml
- Micropipettes: 20μl-200μl, 100μl-1000μl
- 300μl-multipipette

6.2 Reagents

- Ethyl Acetate
- n-hexane
- Sodium hydroxide
- Deionized water

7. Reagents Preparation

Solution 1: 0.8M PBS solution

- Dissolve 45.6g of the $K_2HPO_3 \cdot 12H_2O$ with 250ml of deionized water, mix thoroughly;

Solution 2: 10% NaCl

- Disolve 10g of sodium chloride with 100mL of deionized water;

Solution 3: Wash solution

- Dilute the 20X concentrated wash solution with deionized water in the volume ratio of 1:19, which will be used to rinse the plates. The diluted wash solution can be conserved for one month at 4℃.

8. Sample Preparations

8.1 Notice and precautions before operation:

- Please use one-off tips in the process of experiment and change the tips when absorbing different reagent.
- Make sure that all experimental instruments are clean.
- Treated samples can be stored at 2-8°C for 24h in dark.

8.2 Milk Powder

- Weigh 1.0±0.05g of milk powder into a 10ml polystyrene centrifuge tube, then add 4ml of 10% NaCl (**Solution 2**), shake for 30min to mix completely;
- Transfer 100μl of the solution into a 2ml polystyrene centrifuge tube, add 400μl of the extraction solution (**Kit provided**), vortex for 30s, mix completely;
- Take 50μl of the substrate solution for assay.

8.2 Fresh Milk

- Take 50μl of the substrate solution for assay.

8.3. Milk Products

- Mix 50μl of the milk products and 450μl of the extraction solution (**Kit provided**), vortex for 30s, mix completely;
- Take 50μl of the supernatant of the sample for assay.

9. Assay Process

9.1 Instructions Prior to Beginning Assay

- Ensure that all reagents and microwells are at room temperature (20-25℃). Notice: The antibody solution should be stored at 4℃, which will be used immediately after taking out.
- Return all the rest reagents to 2-8℃ immediately after their use.
- Wash the microwells correctly; this is a vital factor in the reproducibility of the ELISA analysis.
- Avoid direct sunlight during the incubation; use the plate cover provided in the kit to cover the plate.

9.2 Steps in the Assay Process

1. Take all reagents out at room temperature (20-25℃) for more than 30min. Shake gently before use. Notice: The antibody solution should be stored at 4℃, which will be used immediately after taking out.
2. Get the microwells needed out and return the rest into the zip-lock bag at 2-8℃ immediately.
3. The diluted wash solution should be brought to room temperature before use.
4. Number: Number every microwell position and all standards and samples should be run in duplicate. Record the standards and samples positions.
5. Add standard /sample and antibody: Add 50μl of standard solution or prepared sample to corresponding wells. Add 50μl antibody solution (The antibody solution should be stored at 4℃, which will be used immediately after taking out), mix gently by shaking the plate manually and incubate for 60min at 4℃ with cover (or in dark place).
6. Wash: Remove the cover gently and pour the liquid out of the wells and rinse the microwells with 250μl diluted wash solution (solution 5) at interval of 10 seconds for 4-5 times. Absorb the residual water with absorbent paper (the rest air bubble can be eliminated with unused tip).

7. Add enzyme conjugate: Add 100µl enzyme conjugate to each well, mix gently by shaking the plate manually and incubate for 30min at 25℃ with cover. Take out and wash the plate again, following the process in step 6, above.
8. Coloration: Add 50µl solution A and 50µl solution B to each well. Mix gently by shaking the plate manually and incubate for 30 min at 25℃ with cover (see 12.8).
9. Measure: Add 50µl the stop solution to each well. Mix gently by shaking the plate manually and measure the absorbance at 450nm (It's suggested measure with the dual-wavelength of 450/630nm. Read the result within 5min after addition of stop solution)

10. Results

10.1 Calculating the Percentage absorbance

- The mean values of the absorbance values obtained from the standards and the samples are divided by the absorbance value of the first standard (zero standard) and multiplied by 100%.

  Absorbance (%) = $B / B_0 * 100$

  B = the mean absorbance value of each standards or each sample

  B_0 = absorbance value of zero standard

10.2 Drawing a Standard Curve

- To draw a standard curve, the absorbance value of standards as y-axis, semilogarithmic of the concentration of the standards (ppb) as x-axis.
- The nitroimidazoles concentration of each sample (ppb), which can be read from the calibration curve, is multiplied by the corresponding dilution factor of each sample followed, and the actual concentration of sample is obtained.
- Special software has been developed for all data reduction, which can be provided on request.
- Sample dilution factor:
  Milk Powder 20
  Fresh Milk 1
  Milk Products 10

11. Sensitivity, Accuracy and Precision

11.1 Test Sensitivity:

- Overall Sensitivity 0.2ppb

11.2 Detection limit:

- Milk Powder 4ppb
- Fresh Milk 0.2ppb
- Milk Product 2ppb

11.3 Accuracy:

- Milk Powder ..... 80±15%
- Fresh Milk ..... 85±15%

- Milk Product .....90±10%

11.4 Precision:

- C.V. of the ELISA kit .....less than 10%

12. General Instructions

12.1 Temperature of Reagents and Samples

- The mean values of the absorbance values obtained for the standards and the samples will be reduced if the reagents and samples have not been regulated to room temperature (20-25℃). The antibody solution should be stored at 4℃, which will be used immediately after taking out. If the antibody solution is return to room temperature before assay, the OD values will be higher, and the result of the assay will not be right.

12.2 Microwells

- Do not allow microwells to dry between steps to avoid unsuccessful reproducibility and operate the next step immediately after tap the microwells holder.

12.3. Shaking of Reagents

- Shake each reagent gently before use.

12.4. Skin Protection

- Keep your skin away from the stop solution for it is the 0.5M H_2SO_4 solution.

12.5 Out of Date Kits

- Don't use kits that are out of date. Don't exchange the reagents of different batches, or else it will drop the sensitivity.

12.6 General Comments

- Keep the ELISA kits at 2-8℃, do not freeze. Seal rest microwell plates, Avoid straight sunlight during all incubations. Covering the microtiter plates is recommended.

12.7 Special Issues Concerning Solutions and Reagents

- Substrate solution should be abandoned if it turns colors. The reagents may be turn bad if the absorbance value (450/630nm) of the zero standard is less than 0.5 (A450nm<0.5).

12.8 Special Issues Concerning Color

- The coloration reaction need 30min after the addition of solution A and solution B, but you can prolong the incubation time ranges to 35min or more if the color is too light to be determined, never exceed 40min, on the contrary, shorten the incubation time properly.

12.9 Incubation Temperatures

- After adding standard, antibody solution, the incubation temperature is 0-4℃. While after adding enzyme conjugate, substrate A and B, the incubation temperature will be 25℃. Please make sure the temperature is correct during all steps. Higher or lower temperature will lead to experiment failure.

Who we are:

Attogene is a biotechnology company located in Austin, Texas. Our focus is to enhance health and wellness by offering and developing customer focused Life Science Products domestically and internationally.

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- Enhance detection technologies
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