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User's Manual

Vitamin B12 ELISA Kit

REF **DEIASL091**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

This kit can be used for qualitative and quantitative analysis of vitamin B12 in cereals (maize meal, soybean meal, millet flour, rice flour), milk, milk powder.

General Description

The main physiological functions of vitamin B12, which is also named cobalamin are participating and making the bone marrow erythrocyte, avoiding the pernicious anemia, avoiding the damage of the nerves in the brain. The earliest detectable manifestations of infants who lack the vitamin B12 are spirit abnormal, dull expression, less cry and less nois, lags in response, more sleep. Then it will cause the anemia. Some patients has the anaphylaxis for vitamin B12 and even allergic shock. Notice it when use the vitamin B12.

Principles of Testing

This kit is based on indirect-competitive ELISA. The microtiter wells are coated with coupling antigen. Vitamin B12 in the sample competes with the antigen coated on the microtiter plate for the antibody. After the addition of enzyme conjugate, chromogenic substrate is used to show the color. Absorbance of the sample is negatively related to the vitamin B12 concentration in it, After comparing with the standard curve, multiplied by the dilution factor, vitamin B12 residue quantity in the sample can be calculated.

Reagents And Materials Provided

1. Microtiter plate precoated with antigen, 96 wells
2. Standard Solution ×5 bottle (1 mL/bottle)

0µ g/100g, 0.02µg/100g,0.06µg/100g, 0.18µg/100g, 0.54µg/100g

3. Spiking standard solution (1 mL/bottle) 100µg/100g
4. Enzyme conjugate 12 mL
5. Antibody solution 7 mL
6. Substrate A 7 mL
7. Substrate B 7 mL
8. Stop solution 7 mL
9. 20×concentrated wash solution 40 mL
10. Extraction solution 50 mL

Materials Required But Not Supplied

1. Microtiter plate spectrophotometer (450nm/630nm)
2. Shaker
3. Centrifuge

4. Vortex mixer
5. Analytical balance (inductance: 0.01g)
6. Graduated pipette: 10 mL
7. Rubber pipette bulb
8. Polystyrene centrifuge tube: 2 mL, 10 mL
9. Micropipettes: 20-200 µL, 100-1000 µL, 250 µL-multichanel
10. Dipotassium hydrogen phosphate dodecahydrate (K2HPO412H2O) (AR)
11. Sodium chloride (NaCl) (AR)
12. Deionized water

Storage

Storage condition: 2-8°C.

Storage period: 12 months.

Specimen Collection And Preparation

Notice and precautions before operation:

(a)Please use one-off tips in the process of experiment, and change the tips when absorbing different reagent.

(b)Make sure that all experimental instruments are clean.

(c)The sample treated by the method of low detectionlimit tissue sample can't be stored and should be usedimmediately.

(d)The sample treated by other pre-preparation methods can be stored at 4°C avoiding sunlight for 24hours.

Cereal sample (maize meal, soybean meal, millet flour, rice flour)

(1) Homogenize the cereals sample.

(2) Weigh 1.0±0.05 g cereal sample after homogeneity into a 10 mL polystyrene centrifuge tube.

(3) Add 4 mL 0.8M Phosphate buffer solution (solution 1)and shake for 3min. Centrifuge at room temperature(20-25°C/68-77℉) for 5min, at least 3000g;

(4) Take 100 µL upper clean liquid into a 2 mL polystyrene centrifuge tube. Add 400 µL extraction solution and vortex for 30s.

(5)Take 50 µL for assay.

Milk powder

(1) Weigh 1.0±0.05 g milk powder into a 10 mL polystyrene centrifuge tube.

(2) Add 4 mL 10% NaCl solution (solution 2) and shake for 3min.

(3) Take 100 µL milk powder solution into a 2 mL polystyrene centrifuge tube. Add 400 µL extraction solution and vortex for 30s.

(4) Take 50 µL for assay.

Raw milk

(1) Take 50 µL of the raw milk for assay directly.

Finished milk

(1) Take 50 µL milk sample into a 2 mL polystyrene centrifuge tube. Add 450 µL extraction solution and vortex for 30s.

(2) Take 50 µL for assay.

Reagent Preparation

Solution 1: 0.8M Phosphate buffer solution

Dissolve 45.6 g Dipotassium hydrogen phosphate dodecahydrate with 250 mL of deionized water and mix completely.

Solution 2: 10% NaCl solution

Dissolve 10.0 g NaCl with 100 mL of deionized water and mix completely.

Solution 3: Wash solution

Dilute 20×concentrated wash solution with deionized water in the volume ratio of 1:19, which will be used to wash the plates. This diluted solution can be stored for 1 month at 4°C.

Assay Procedure

Notice before assay:

1. Make sure all reagents and microwells are all at room temperature (20-25°C).
2. Return all the rest reagents to 2-8°C immediately after used.
3. Washing the microwells correctly is an important step in the process of assay; it is the vital factor to the reproducibility of the ELISA analysis.
4. Avoid the light and cover the microwells during incubation.

Assay Steps:

1. Take all reagents out at room temperature (20-25°C) for more than 30min, shake up before use.
2. Get the microwells needed out and return the rest into the zip-lock bag at 2-8°C immediately.
3. The concentrated wash solution and extraction solution should be rewarmed 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 solution/sample, and antibody solution: add 50 µL standard solution or prepared sample to corresponding wells. Add 50 µL antibody solution, mix gently by shaking the plate manually and incubate for 30min at 25°C with cover.
6. Wash: remove the cover gently and pour the liquid out of the wells and rinse the microwells with 250 µL wash solution (solution 3) at interval of 10s 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 100 µL enzyme conjugate to each well and shake gently. Incubate for 30min at

25°C with cover. Then repeat the step 6.

8. Coloration: add 50 µL solution A and 50 µL solution B to each well. Mix gently by shaking the plate manually and incubate for 15min at 25°C with cover.
9. Measure: add 50 µL stop solution to each well. Mix gently by shaking the plate manually and measure the absorbance at 450nm against an air blank (It's suggested that measure with the dual-wavelength of 450/630nm. Read the result within 5min after addition of stop solution. We can also observe by sight without stop solution in short of the ELISA reader)

Calculation

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/B0*100%

B ——absorbance of standards or samples

B0 ——absorbance of zero standard

Typical Standard Curve

1. To draw a standard curve: The absorbance value of standards as y-axis, semi-logarithmic of the concentration of the standards (ppb) as x-axis.
2. The vitamin B12 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.

Notice:

Special software has been developed for result calculation, which can be provided on request.

Sample dilution factor:

Cereals: 20

Milk powder: 20

Raw milk: 1

Finished milk: 10

Precision

Variation coefficient of the ELISA kit is less than 10%.

Detection Limit

Cereals.....0.4µg/100g

Milk powder.....0.4µg/100g

Raw milk.....0.02µg/100g

Finished milk.....0.2μg/100g

Sensitivity

0.02μg/100g

Specificity

Vitamin B12……100%

Recovery

Cereals.....85%±15%

Milk powder.....80%±10%

Raw milk..... 85%±15%

Finished milk.....90%±10%

Precautions

1. 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°C) or the room temperature is lower than 20°C.
2. Do not allow microwells to dry between steps to avoid unsuccessful reproducibility and operate the next step immediately after tap the microwells holder.
3. Shake each reagent gently before using.
4. Keep your skin away from the stop solution for it is the 0.5M H2SO4 solution.
5. Don't use the kits out of date. Don't exchange the reagents of different batches, or else it will drop the sensitivity.
6. Keep the ELISA kits at 2-8°C, do not freeze. Seal rest microwell plates, Avoid straight sunlight during all incubations. Covering the microtiter plates is recommended.
7. Substrate solution should be abandoned if it turns colors. The reagents may turn bad if the absorbance value (450/630nm) of the zero standard is less than 0.5 (A450nm<0.5).
8. The coloration reaction needs 15min after the addition of solution A and solution B; But you can prolong the incubation time ranges to 20min or more if the color is too light to be determined., never exceed 25min, On the contrary, shorten the incubation time properly.
9. The optimal reaction temperature is 25°C. Higher or lower temperature will lead to the changes of sensitivity and absorbance values.