Vitamin B12 ELISA Kit

Enzyme Immunoassay for the quantitative determination of Vitamin B12 in food

Catalog number: ARG80816

Package: 96 wells

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INTRODUCTION

Vitamin B12 as a trace element belongs to the biologi-cally important chelate formers. The basic unit con-sists of a corrin ring with cobalt as a central atom. Cobalt is sixfold coordinated by four nitrogen atoms, one cyanide and a dimethylbenzimidazol group. Vitamin B12 forms a stable complex, which is absorbed in the lower part of the small intestine, with the so-called intrinsic factor present in the gastric juice. A lack of vitamin B12 can lead among other things to pernicious anemia. This disease is not generated by an insufficient supply of vitamin B12, but by the ab-sence of intrinsic factor. A pernicious anemia can be treated by a high dosage of vitamin B12.

The existing detection procedures are mainly microbiological methods, but also HPLC and thin-layer chromatography, all of which are associated with a high amount of time and instrumentation.

With the present test kit it is possible, to determine vitamin B12 quantitatively in vitaminated food in a significantly faster way (2.5 to 4 hours inclusive sam-ple pretreatment) compared with a conventional microbiological assay (24 to 48 hours).

PRINCIPLE OF THE ASSAY

This assay employs the competitive quantitative enzyme immunoassay technique. An antibody directed against Vitamin B12 is coated on the surface of a microtiter plate. Vitamin B12 containing samples or standards and a vitamin B12-HRP conjugate are given into the wells of the microtiter plate. Enzyme labeled and free vitamin B12 compete for the antibody binding sites.

After one hour incubation at room temperature, the wells are washed with diluted washing solution to remove unbound material. A substrate solution is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is inhibited by the addition of a stop solution, and the color turns yellow. The yellow color is measured at 450 nm. The concentration of vitamin B12 is indirectly proportional to the color intensity of the test sample.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	12 strips x 8-well	4°C
HRP-Vitamin B12 Conjugate antibody	6 ml (ready to use)	4°C
Standards A-F (0,0.4, 1, 4, 10, 40 ng/ml)	6 X 0.5 ml (ready to use)	4°C
Sample dilution buffer	2 X 60 ml (ready to use)	4°C
10x Wash Buffer	30 ml	4°C
TMB substrate	15 ml	4°C (Protect from light)
STOP solution	15 ml	4°C
Plastic foils	2	

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Briefly spin down the HRP-Vitamin B12 conjugate before use.
- If crystals are observed in the 10X Wash buffer and Sample diluent buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
- Samples contain azide cannot be assayed.

SAMPLE COLLECTION & STORAGE INFORMATION

The vitamin is extracted from the sample by double-distilled water. After the dissolution, the pH is adjusted by 1 M caustic soda solution or 1 M hydrochloric acid to 6-7. Afterwards potential turbid matter is precipitated by Carrez I (150 g/l Potassiumhexacyanoferrate(II)-3-hydrate) and Carrez II (300 g/l Zincsulfate-7-hydrate). The extract is filled up to a defined volume and is centrifuged.

Samples which are difficult to dissolve in cold water can be brought in solution by gentle warming. After the centrifugation, the samples are further diluted by the supplied sample diluent. To exclude interfering matrix or pH effects, a minimal dilution of 1 in 5 should be followed. We recommend a dilution to 1-10 ng/ml, in order to obtain an optimal accuracy during the measurement.

Grain products normally contain low concentrations of vitamin B12. In order to avoid high dilutions, the sample can be extracted directly by sample diluent instead of double-distilled water.

Multivitamin Tablets and Capsules

The tablets and capsules are dissolved in double-distilled water, and the pH value is adjusted to 6-7. Then 0.5 ml each of Carrez I and Carrez II are added, and the solution is filled up to a defined volume by double-distilled water. The solid matter is separated by centrifugation, and the upper phase is further diluted by sample diluent. To dissolve the capsules, heating to 30-40°C is recommended.

Multivitamin Juices

The juice is adjusted to pH 6-7, 0.5 ml each of Carrez I and Carrez II are added, and the solution is filled up to a defined volume by double-distilled water. The solid matter is separated by centrifugation, and the upper phase is further diluted by sample diluent.

Multivitamin Jam

The jam is homogenised in a mixer, and approximately 8 grams are extracted by double-distilled water, the pH is adjusted to 6-7 and 0.5 ml each of Carrez I and Carrez II are added. Afterwards the solution is filled up to a defined volume by double-distilled water. The solid matter is separated by centrifugation, and the upper phase is further diluted by sample diluent.

Grain Products (Corn Flakes and Muesli)

3-5 grams of sample are homogenised by a mortar or a mixer, extracted by double-distilled water, the pH is adjusted to 6-7, and 0.5 ml each of Carrez I and Carrez II are added. Afterwards the solution is filled up to a defined volume by double-distilled water. The solid matter is separated by centrifugation, and the upper phase is further diluted by sample diluent. Grain products normally contain low concentrations of vitamin B12. In order to avoid high dilutions, the sample can be extracted directly by sample diluent instead of double-distilled water.

Multivitamin Sweets

The sweets are dissolved by gentle heating (if necessary) in double-distilled water, the pH is adjusted to 6-7, and 0.5 ml each of Carrez I and Carrez II are added. Afterwards the solution is filled up to a defined volume by double-distilled water. The solid matter is separated by centrifugation, and the upper phase is further diluted by sample diluent.

Milk

5 mL of a fresh milk sample (full-cream milk or skim milk) are pipetted into a test tube and refrigerated for 30 minutes at 2-8°C. Afterwards the sample is centrifuged for 10 min at 3000 g. The upper fat layer is aspirated and discarded.

The remaining aqueous layer is diluted 1:5 in sample diluent.

Dry Milk Instant Formula

10 g of dry milk instant formula are suspended in 25 ml PBS and filled up to 50 ml. The mixture is vortexed intensely for 10 min and heated for 3 min in boiling water afterwards. After cooling to 20-25°C it is centrifuged for 10 min at 3000 g. The upper fat layer is aspirated and discarded. The remaining aqueous layer is diluted 1:5 in sample diluent.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 10X wash buffer into distilled water to yield 1X wash buffer.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Pipet 50 µl standards or prepared samples in duplicate into the appropriate wells of the microtiter plate. Immediately add 50 µl HRP-Vitamin B12 into each well.
3. Cover the microtiter plate with a plastic foil and incubate for 60 minutes at RT.
4. Aspirate each well and wash, repeating the process 2 times for a total 3 washes. Wash by filling each well with 1X wash buffer (350 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash,

remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.

5. Add 100 µl of TMB mixture to each well. Incubate for 20 minutes at room temperature in dark.
6. Add 100 µl of Stop Solution to each well.
7. Read the OD with a microplate reader at 450 nm immediately.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

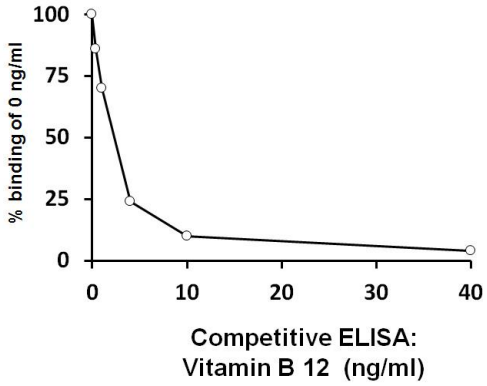
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results. The diluted samples must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The sensitivity of the Vitamin B12 ELISA is 0.3 ng/mL (based on the standard curve).

Specificity

Cross-reactivity	Relative to Vitamin B12 (=100%)
Hydroxycobalamine	29%

Intra-assay

The CV value of intra-assay precision was 3%.

Recovery

98%