ID-Vit® Vitamin B₁₂

Microbiological test kit for the determination of vitamin B₁₂ in serum using a Lactobacillus delbrueckii subsp. lactis coated microtitre plate
For use in human and veterinary medicine and in research

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1. INTENDED USE

**ID-Vit® Vitamin B\textsubscript{12}** is a microtiter plate test kit based on a microbiological assay which measures the total Vitamin B\textsubscript{12} content in serum. The test kit contains all required reagents, e.g. standard, medium and microtiter plate coated with a specific microorganism, sufficient for 96 determinations including standard curves. An ELISA reader is required for evaluation of the Vitamin B\textsubscript{12} content. For use in human and veterinary medicine and in research. The test kit is for in vitro diagnostic use only.

2. INTRODUCTION

Vitamin B\textsubscript{12} (Cobalamin), a collective term for a group of various substituted corrinoides with cobalt as the central atom, is found free and also protein-bound in food. The protein-bound form is degraded by pancreatic protease, releasing free B\textsubscript{12} which binds to intrinsic factor, a protein secreted by gastric parietal cells of the stomach mucosa. The Cobalamin-Intrinsic-Factor-Complex is bound to mucous membrane cells of the stomach in the ileum and absorbed by the cells. In the case of high doses, a diffusion of the complex also takes place. Vitamin B\textsubscript{12} is bound to the protein Transcolbalamin II (TC-II) within the cells. TC-II serves as a transport protein for Vitamin B\textsubscript{12} in the circulation system.

Vitamin B\textsubscript{12} is involved in the metabolism process as a co-enzyme, and plays an important role in the formation of the blood, the development of the nervous system and the regeneration of the mucous membranes. In addition, there is a direct relationship to the formation of folic acid because methylcobalamin is involved in the transfer of methyl groups for the synthesis of methionin from homocystein.

**Vitamin B\textsubscript{12} -Deficiency**

Vitamin B\textsubscript{12} deficiency is seldom caused by dietary factors. In most cases, it results from a resorption disorder of the intestines or defective development of intrinsic factor. Since vitamin B\textsubscript{12} resorption could be reduced up to 50% in the elderly, an increased supplement is recommended. Pregnant women with a lacto-vegetarian diet are also recommended to increase the intake because their liver vitamin B\textsubscript{12} stores may be exhausted.

The classical vitamin B\textsubscript{12} deficiency disease is pernicious anemia. In the early stages of the disease, vitamin B\textsubscript{12} deficiency symptoms are manifested as weariness, palpitations, pallor or jaundice.
Indications for Vitamin-$B_{12}$-determination

- Megaloblastic (pernicious) anemia
- Hyperhomocysteinaemia (patients on dialysis, old people)
- Homocysteinurie
- Peripheral neuropathy
- Patients with CED, gastritis, gastrektomy, gluten intolerance or intestinal resorption disorders, pancreatic insufficiency
- Patients with thrombosis
- Alcoholism
- Chronic liver and kidney disease
- Vitamin $B_{12}$ deficiency from diet (vegan vegetarians)
- Pregnancy and lactation

3. PRINCIPLE OF THE TEST

The serum is pre-treated and diluted with a buffer mixture, and then samples were transferred in the wells of a microtiter plate [PLATE] coated with Lactobacillus delbrueckii subsp. lactis. The addition of vitamin $B_{12}$ in either standards [STD] or samples gives a vitamin $B_{12}$-dependent growth response until vitamin $B_{12}$ is consumed. After incubation at $37^\circ C$ for 44 - 48 h, the growth of Lactobacillus plantarum is measured turbidimetrically at 610 - 630 nm (alternatively at 540 - 550 nm) in an ELISA-reader and a standard curve is generated from the dilution series. The amount of vitamin $B_{12}$ is directly proportional to the turbidity.
4. MATERIAL SUPPLIED

<table>
<thead>
<tr>
<th>Catalog No</th>
<th>Label</th>
<th>Kit Components</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>KIF012MTP</td>
<td>PLATE</td>
<td>One Lactobacillus delbrueckii subsp. lactis-precoated microtiter plate, ready to use</td>
<td>12 x 8 wells</td>
</tr>
<tr>
<td>KIF012SO</td>
<td>SOL</td>
<td>Sample preparation buffer 5 ml, ready to use</td>
<td>4 x</td>
</tr>
<tr>
<td>KIF012STAB</td>
<td>STAB</td>
<td>Stabilizer</td>
<td>4 x</td>
</tr>
<tr>
<td>KIF012DI</td>
<td>DIL</td>
<td>Water 30 ml</td>
<td>4 x</td>
</tr>
<tr>
<td>KIF012ME</td>
<td>ASYMED</td>
<td>Vitamin B₁₂ assay medium</td>
<td>4 x</td>
</tr>
<tr>
<td>KIF012ST</td>
<td>STD</td>
<td>Vitamin B₁₂ standard</td>
<td>4 x</td>
</tr>
<tr>
<td>KIF012KO</td>
<td>CTRL</td>
<td>Control Vitamin B₁₂</td>
<td>4 x</td>
</tr>
<tr>
<td>KIF012FO</td>
<td>FOL</td>
<td>Cover plastic foil</td>
<td>4 x</td>
</tr>
<tr>
<td>KIF012FR</td>
<td>FRA</td>
<td>Replacement holder for 96-well plates</td>
<td>1 x</td>
</tr>
</tbody>
</table>

5. MATERIAL REQUIRED BUT NOT SUPPLIED

- Incubator with a dark incubation chamber, 37 °C
- Water bath (90°C - 100°C)
- ELISA-Reader 610 - 630 nm (540 - 550 nm)
- Micropipette 20 - 200 µl
- Micropipette 100 - 1000 µl
- Micropipette tips to deliver 20 - 200 µl and 100 - 1000 µl, sterile
- Pipettes of 5 and 10 ml
- 1,5 - 2 ml reaction vials, sterile
- 0,2 µm sterile polyethersulfone filter with a sterile tip
- 15 ml centrifugal tubes, sterile (e.g. Falcon tubes)
- Biocentrifuge (10 000 x g)
6. **PREPARATION AND STORAGE OF REAGENTS**

- Store test kit / reagents at 2-8°C.
- Prepare reagents freshly and use immediately after preparation. Discard remaining unused reagents and waste in accordance with country, federal, state, and local regulations.
- Put unused reagents (standard, medium) in the test kit and store at 2-8°C.
- Store unused strips in the original package with dry bag securely closed at 2-8°C to prevent contamination or moisture exposure.
- No warranty can be given after the expiry date (see label of test package).
- To run assay more than once, ensure that reagents are stored at conditions stated on the label. Prepare only the appropriate amount necessary for each assay. The kit can be used up to 4 times within the expiry date stated on the label.

7. **PRECAUTIONS**

- As the test is based on a microbiological method, the general guidelines for sterile work must be observed as far as possible, (work in a sterile bench, PCR-Hood, use of sterile instruments or equipment).
- GLP (Good Laboratory Practice)-guidelines should be observed.
- Water quality is extremely important. Only the water delivered with the test kit [DIL] should be used for medium dilution [ASYMED], standard [STD] reconstitution as well as for sample preparation.
- It is essential to run a standard curve for each separate assay.
- It is recommended to run a duplicate standard [STD] curve as well as a sample analysis.
- If a higher dilution results in a higher measured value, inhibitors like antibiotics might be present.
- Reagents should not be used beyond the expiration date shown on kit label.
- Wear gloves during the test.
- Used microtiter plates [PLATE] and materials that have been in contact with patient’s samples should be handled and disposed as potentially infectious.
- Signs for reagent damage: The highest standard should have an absorption higher than 0.6 Extinktion units (A630nm > 0.6)
8. **SAMPLE PREPARATION**

Notes

- Patient serum is used for analysis.
- Original samples should be kept light-protected at 2-8°C until measurement. The samples are stable for 8 hours at 2-8°C in the dark. For longer storage, samples should be frozen and kept at -20°C.
- Hemolytic samples may give erroneous results and should not be used for analysis. Lipemic samples should be centrifuged at 13 000 x g before assaying to obtain fat free serum as far as possible.
- Samples should be centrifuged (5 min at 10000 g) prior to measurement and the resulting supernatant used in the test.

8.1 **Sample pretreatment**

Add 4 ml sample preparation buffer [SOL] to the bottle with the stabilizer [STAB]. Mix well. Add 75 µl serum sample or control [CTRL] to 300 µl of this solution, mix, heat to 95°C for 30 min and then cool quickly. Afterwards, centrifuge for 10 min at 10000 g.

8.2 **Sample dilution**

Take 100 µl from the supernatant of the treated serum sample, add 400 µl water [DIL] and mix. The sample treatment and dilution results in a final dilution of 1:25 (= sample dilution factor).

9. **ASSAY PROCEDURE**

Procedural notes

- Quality control guidelines should be observed.
- Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the test.
- The assay should always be performed according the enclosed manual.
9.1 Test preparations

Take as many microtiter strips as needed from kit. Return unused strips and any unused test kit component to the original foil bag, reseal them together with the desiccant provided, and put in the refrigerator. Bring all necessary reagents to room temperature.

Water [DIL] for medium [ASYMED] and standard [STD]

Push the lid up, pull it back to the rim of the glass and then remove the entire seal by turning.

Assay medium [ASYMED]

- The medium must be freshly prepared before each test.
- Take the dry bag out of medium vial [ASYMED] by tweezers, shake off and discard.
- Add 10 ml of water [DIL] to the assay medium [ASYMED], securely close the bottle and shake well. The amount is sufficient for 6 strips.
- Heat the bottle with medium [ASYMED] in a water-bath at 90 - 100 °C for 5 min, while shaking well at least twice. It is important to make sure that the medium bottle [ASYMED] is firmly closed at all times.
- Quickly cool the medium bottle [ASYMED] to under 30 °C.
- Filter 10 ml medium [ASYMED] steriley with a 0.2 µm filter in a centrifuge test tube (e.g. 15 ml, Falcon).

Control [CTRL]

- The control must be freshly prepared before the test.
- Open the bottle of control [CTRL], remove seal. Dispose of screw-top lid and seal.
- Add 0.3 ml water [DIL] from the test kit to the control bottle [CTRL], close the bottle and dissolve by vortexing the bottle (= control).
- Treat the control afterwards as the sample is treated.
- Pipette 150 ml of the reconstituted control [CTRL] into each well. We recommend to run a duplicate.
- For the concentration of the control [CTRL] please see control specification.

Standard [STD]

Before the test, freshly prepare the standard curve solutions:

- Open the bottle of standard [STD], place the screw-top lid upside-down on the work bench.
- Add x ml (x = see QS test kit data sheet) water [DIL] from the test kit to the standard bottle [STD], close the bottle and shake (= standard concentrate).
- Add water [DIL] into 6 sterile reaction vials (capacity 1.5 – 2.0 ml) and then pipet the standard concentrate to the vials. Prepare a standard curve using the following scheme:
### 9.2 Test Initiation

- Take as many microtiter strips as needed from the kit and put them in the second microtiter strip holder [FRA]. Return unused strips to the original foil bag, reseal them together with the desiccant provided, and store at 2-8°C to prevent contamination or moisture exposure.
- A medium solution is sufficient for 6 strips.
- Put 150 µl Vitamin \( B_{12} \) assay medium [ASYMED] in the cavities.
- Add 150 µl standard [STD], respectively, sample in the cavities. Pre-rinse the pipette tip with standard and sample solution respectively.
- Carefully seal the plate with plastic foil [FOL]. Important: the cavities must be made airtight by pressing down with the hand!
- Keep at \( 37^\circ C \) for 48 hrs in an incubator.

### 9.3 Measurement

- Securely press the foil [FOL] down with the hand.
- Upturn the plate [PLATE] onto a tabletop and shake the germination well.
- Turn the plate [PLATE] over again and carefully remove the foil [FOL], beginning with the upper right corner and pulling diagonally backwards at an angle of 180°. During this fix the strips in the frame with your hand because the foil is highly adhesive.
- Remove air bubbles in the cavities using a pipette tip or a needle.
- Read turbidity in an ELISA-Reader at E 610 - 630 nm (alternatively at 540 - 550 nm).

<table>
<thead>
<tr>
<th>Vitamin ( B_{12} ) [ng / l]</th>
<th>Water [DIL] [µl]</th>
<th>+</th>
<th>Standard [STD] [µl]</th>
<th>=</th>
<th>Total volume [µl]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank:</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>=</td>
<td>700</td>
</tr>
<tr>
<td>Standard 1:</td>
<td>700</td>
<td>+</td>
<td>50</td>
<td>=</td>
<td>750</td>
</tr>
<tr>
<td>Standard 2:</td>
<td>400</td>
<td>+</td>
<td>100</td>
<td>=</td>
<td>500</td>
</tr>
<tr>
<td>Standard 3:</td>
<td>350</td>
<td>+</td>
<td>150</td>
<td>=</td>
<td>500</td>
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<tr>
<td>Standard 4:</td>
<td>300</td>
<td>+</td>
<td>200</td>
<td>=</td>
<td>500</td>
</tr>
<tr>
<td>Standard 5:</td>
<td>200</td>
<td>+</td>
<td>300</td>
<td>=</td>
<td>500</td>
</tr>
</tbody>
</table>
**Please note**
- After 48 hrs incubation time, the microtiter plate [PLATE] may be stored for a maximum of 48 hrs in the refrigerator before measuring the turbidity.
- To prevent time-loss through public holidays or weekends, the microtiter plate [PLATE] may also be evaluated after 60 hrs incubation.

### 10. EVALUATION OF RESULTS

We recommend to use the „4-Parameter-algorithm to calculate the results. The sample dilution factor should be considered for data evaluation.

#### Serum

Vitamin $B_{12}$ in ng/l = Value from the standard curve x sample dilution factor

**Reference Value**

\[
\text{Vitamin } B_{12} \text{ (n = 83): } 25 - 500 \text{ ng/L} \quad (= 18.4 - 500 \text{ pmol/L})
\]

**Please note:** A concentration range of 150 - 1350 ng/L Vitamin $B_{12}$ is covered at a sample dilution 1:4.

We recommend each laboratory to develop its own normal range as normal ranges depend on the choice of patient collective. The values mentioned above are only for orientation and can deviate from other published data.

### 11. PERFORMANCE CHARACTERISTICS

**Precision and reproducibility**

<table>
<thead>
<tr>
<th>Intra Assay (n = 21)</th>
<th>Vitamin $B_{12}$ [ng/L]</th>
<th>CV [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>294</td>
<td>5.38</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Inter-Assay (n = 3)</th>
<th>Vitamin $B_{12}$ [ng/L]</th>
<th>CV [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>285</td>
<td>8.0</td>
</tr>
</tbody>
</table>
Recovery

Samples from 3 patients were spiked with Vitamin B$_{12}$ and analyzed. The mean values are shown below:

\[ n = 5 \]

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean value measured in original sample [ng/l]</th>
<th>Spike [ng/l]</th>
<th>Vitamin B$_{12}$ expected [ng/l]</th>
<th>Vitamin B$_{12}$ measured [ng/l]</th>
<th>Recovery Rate [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>566.58</td>
<td>187.5</td>
<td>754.08</td>
<td>726.36</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td></td>
<td>375.0</td>
<td>941.58</td>
<td>908.21</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td><strong>Recovery rate in total [%]</strong></td>
<td></td>
<td></td>
<td></td>
<td><strong>88</strong></td>
</tr>
<tr>
<td>B</td>
<td>481.3</td>
<td>187.5</td>
<td>668.8</td>
<td>681.45</td>
<td>107</td>
</tr>
<tr>
<td></td>
<td></td>
<td>375.0</td>
<td>856.3</td>
<td>929.50</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td><strong>Recovery rate in total [%]</strong></td>
<td></td>
<td></td>
<td></td>
<td><strong>114</strong></td>
</tr>
</tbody>
</table>
n = 5

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean value measured in original sample [ng/l]</th>
<th>Spike [ng/l]</th>
<th>Vitamin B$_{12}$ expected [ng/l]</th>
<th>Vitamin B$_{12}$ measured [ng/l]</th>
<th>Recovery Rate [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>526.44</td>
<td>187.5</td>
<td>713.94</td>
<td>762.23</td>
<td>126</td>
</tr>
<tr>
<td></td>
<td></td>
<td>375.0</td>
<td>901.44</td>
<td>845.02</td>
<td>85</td>
</tr>
</tbody>
</table>

Recovery rate in total [%] 105

12. REFERENCES


13. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- This assay was produced and put on the market according to the IVD guidelines of 98/79/EC.
- Test components contain organic solvents. Contact with skin or mucous membranes must be avoided.
- All reagents in the test package are for in vitro diagnostic use only.
- Reagents should not be used after the date of expiry stated on the label.
- Single components with different lot numbers should not be mixed or exchanged.
- Guidelines for medical laboratories should be observed.
- Incubation time, incubation temperature and pipetting volumes of the different components are defined by the producer. Any variations of the test procedure that are not coordinated with the producer may influence the results of the test. ifp Institute for Product Quality GmbH can, therefore, not be held reliable for any damage resulting from this.
Used symbols:

- Temperature limitation
- Catalogue Number
- In Vitro Diagnostic Medical Device
- Contains sufficient for \(<n>\) tests
- Manufacturer
- Use by
- Lot number