Vitamin B1 (Thiamin)

Mikrobiologischer Mikrotiterplatten-Test zur quantitativen Bestimmung von Vitamin B1 (Thiamin)

Microbiological microtiter plate test to quantitate vitamin B1 (thiamine)

Art. No.: P1006

In vitro Test
Lagerung bei 2 - 8 °C
Storage at 2 - 8 °C (35.6 - 46.4 °F)

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VitaFast® Vitamin B1 (Thiamine)

**Brief information**

Easy to use microbiological microtiter plate test for the quantification of total vitamin B1 (enriched and natural vitamin B1) in food, animal feed and pharmaceutical products. All required reagents and standard are contained in the test kit. The kit is sufficient for 96 determinations incl. standard. Evaluation requires a microtiter plate reader (610 - 630 nm alternatively at 540 - 550 nm).

**Sample preparation**

Liquid samples (added vitamin B1):
sterile filtration and dilution

Solid samples (added vitamin B1):
sample homogenization, extraction, centrifugation and dilution

Liquid and solid samples (native and added vitamin B1):
sample homogenization, enzymatic treatment, extraction, centrifugation and dilution

Time requirement:
Test conduction .......................approx. 60 min
Evaluation.................................2 min

Incubation: 44 - 48 h in the dark at 37 °C (98.6 °F)

Standard range: 0.012 - 0.060 mg / 100 g (ml)

Recovery: 90 - 105 %

Intra-assay CV for standards: < 10 %
Inter-assay CV for standards: < 10 %
1. Principle of the test

The VitaFast® Vitamin B1 (Thiamine) microtiter plate test is a microbiological method for the quantitative determination of total vitamin B1 (added and natural vitamin B1) in food, animal feed and in pharmaceutical products. The microbiological test system is in accordance with international norms.

Vitamin B1 is extracted from the sample and the extract is diluted. The vitamin B1 assay - medium and the diluted extract are pipetted into the wells of a microtiter plate which is coated with Lactobacillus fermentum. The growth of Lactobacillus fermentum is dependent on the supply of vitamin B1. Following the addition of vitamin B1 as a standard or as a compound of the sample, the bacteria grow until the vitamin is consumed. The incubation is done in the dark at 37 °C (98.6 °F) for 44 - 48 h.

The intensity of metabolism or growth of Lactobacillus fermentum in relation to the extracted vitamin B1 is measured as turbidity and compared to a standard curve. The measurement is done using a microtiter plate reader at 610 - 630 nm (alternatively at 540 - 550 nm).

2. Reagents provided

Each test kit contains sufficient materials for 96 determinations incl. standards. Each test kit contains:
1 x microtiter plate with 96 wells, coated with Lactobacillus fermentum
3 x redist., sterile water (30 ml) for the preparation of assay - medium, standards and dilution of extracted samples
3 x vitamin B1 assay - medium (solid)
3 x vitamin B1 (thiamine hydrochloride) standard (solid)
3 x adhesive foil (1 complete and 2 halves of adhesive foils, sufficient for 3 test runs)
1 x additional holder for microtiter strips

Remark: after the expiry date no guarantee of quality
3. Required reagents and instruments, not provided

- **sterile bench** (sterile working is recommended)
- **microtiter plate reader** 610 - 630 nm (540 - 550 nm)
- incubator with dark chamber, 37 °C (98.6 °F)
- water bath heatable to 95 °C (203 °F)
- autoclave
- pH meter
- centrifuge > 8,000 x g (in the case that the extracted sample cannot be filtrated)
- sterile tips for graduated micropipette 20 - 200 µl and 100 - 1000 µl
- sterile graduated centrifuge vials with screw cap (15 and 50 ml) and sterile vials 1.5 or 2.0 ml
- 500 ml screw glass jar, 100 and 1000 ml volumetric flask, 100 ml beaker
- sterile filters polyethersulfon 0.2 µm with syringe
- redist. or deionized water for sample extraction
- HCl 1.0 mol / l and 0.1 mol / l
- NaOH concentrated (40 g NaOH ad 100 ml redist. or deionized water)
- NaOH 2 mol / l (8 g solid NaOH ad 100 ml redist. or deionized water)

Reagents for the determination of native vitamin B1

- taka diastase, aspergillus oryzae (e. g. Fluka 86250)
- phosphatase, Acid, Type II: from potato (e. g. Sigma P3752)
- H₂SO₄ 0.1 mol / l
- sodium acetate 2.5 mol / l (solve 34 g sodium acetate trihydrate, e. g. Fluka 71190, in 100 ml redist. or deionized water)
- citrate buffer pH 4.5 (weigh 1.5 g citric acid monohydrate (e.g. Roth 5110.3) in a 100 ml beaker with magnetic stirrer; solve the citric acid with about 50 ml dist. or. deionized water under stirring; thereafter add 12 ml NaOH 1 mol / l (or 0.48 g NaOH); the pH should be 4.5 (correct with HCl 0.1 mol / l); transfer the solution quantitatively with dist. or deionized water in a 100 ml volumetric flask and fill up with dist. or deionized water to the mark; the buffer can be stored 3 days at 2 - 8 °C (35.6 - 46.4 °F)

4. Warning and precautions for the user

- the assay - medium could evoke irritations of mucosa, eyes and skin
- after running the test the strips used must be disposed of according to regulations (e.g. autoclaved)
5. Storage instructions

Store the kit / reagents at 2 - 8 °C (35.6 - 46.4 °F).

Use the prepared reagents (standard, medium) directly and reject them after the assay.

6. Sample preparation

For the determination of added vitamin B1 in nutrient enriched solid samples, a hot acid extraction is usually sufficient. Liquid samples can be used directly after sterile filtration and dilution with sterile water from the test kit.

For the determination of the total vitamin B1 (native and added) content, the sample has to be treated with enzyme.

Samples should be stored protected from light at 4 °C (39.2 °F). Standard and samples should be run in triplicate. Unknown sample matrices should be analyzed with two dilutions of the sample extract. Sample extracts have to be used within one day and should be stored in the dark until analyzing.

Sample extraction is carried out with 1 g (ml) homogenized sample in 40 ml redist. or deionized water or extraction solution. This equals a sample extraction dilution factor of 40. This factor is already included in the standard curve (see Quality Assurance Certificate). For low vitamin B1 concentrations a sample weight of up to 5 g (ml) can be used (this has to be considered in the evaluation).

Only sterile sample extracts or sterile dilutions thereof should be pipetted onto the microtiter plate. Dilutions have to be prepared with sterile water from the test kit. Therefore after the sample extraction sterile working conditions and sterile consumables are necessary. A sterile filtration of the sample or the sample extract is always necessary for:

– samples like fruit juices and fitness drinks, which are not heated during sample extraction (except when the sample is heated 30 min at 95 °C (203 °F) in a water bath)
– samples containing herbs and spices as well as honey and tea
– vitamin mixes, premixes, tablets (highly enriched samples, see 6.3) (except when the sample is heated 30 min at 95 °C (203 °F) in a water bath)
– samples with low vitamin concentrations that are highly coloured (the filtration step eliminates the colouring)
– if filtration is not possible due to solid particles or due to cloudiness, centrifugation should be carried out before the sterile filtration step (greater than 8000 x g for 5 min)

The sterile filtration of the sample is not necessary if the sample extraction is carried out at 95 °C (203 °F) for 30 min. Nevertheless the extracted samples have to be diluted with sterile water from the test kit (the assay - medium has to be always filtered).

Example for the dilution factors of the sample extract

Solid sample with a labeled concentration of 2.0 mg active vitamin B1 / 100 g

The dilution of the extract should be in the middle of the standard curve. The test is calibrated with thiamine hydrochloride. The active vitamin (thiamine) is labeled. Therefore, the expected concentration is converted with factor 1.27 into thiamine hydrochloride and divided by standard 3.

**Calculation:**

2.0 mg active vitamin B1 / 100 g x 1.27 = 2.54 thiamine hydrochloride / 100 g

2.54 mg thiamine hydrochloride / 0.036 mg = 71
→ dilution factor 70 (1: 70)

**Dilution steps:**

a) 1 : 10 → 0.1 ml sample extraction solution + 0.9 ml sterile water from the test kit
b) 1 : 7 → 0.1 ml from a) + 0.6 ml sterile water from the test kit

6.1. Added vitamin B1 in liquid samples (multivitamin juices, fitness drinks)

Add 1 ml sample into a 50 ml sterile centrifuge vial and fill up exactly to 40 ml with redist. or deionized water. Thereafter shake, sterile filter (or heat the sample 30 min at 95 °C in a water bath, thereafter chill down quickly below 30 °C (86 °F)) and, depending on the concentration range, further dilute in 1.5 ml (or 2.0 ml) sterile reaction vials with sterile water from the test kit.
6.2. Added vitamin B1 in fruit gums and candies

Weigh about 15 - 20 g fruit gums or candies in a 50 ml sterile centrifuge vial, add about 40 ml redist. or deionized water and solve the sample at 95 °C (203 °F) in a water bath. Chill down quickly to below 30 °C (86 °F), transfer the extraction solution with redist. or deionized water quantitatively into a 100 ml volumetric flask and fill up to the mark with redist. or deionized water. Transfer the volume corresponding to 1 g of test material into a 50 ml centrifuge vial. Fill up to 40 ml with redist. or deionized water, shake, sterile filter (or heat the sample 30 min at 95 °C in a water bath, thereafter chill down quickly below 30 °C (86 °F)) and, depending on the concentration range, further dilute in 1.5 ml (or 2.0 ml) sterile reaction vials with sterile water from the test kit.

**Example:** sample weight 17 g fruit gums

calculation: 100 ml / 17 g sample weight = 5.88 ml / g

1 g sample is contained in 5.88 ml. Transfer 5.88 ml into a 50 ml centrifuge vial and continue as described above.

6.3. Added vitamin B1 in capsules, pills and vitamin mixes

First the capsule or pill weight has to be determined (average of 5 - 10 capsules or pills). Then pills are homogenized in a mortar or mixer. Capsules are cut open and extracted.

6.3.1 Sample preparation with 1 g sample size and pre-extraction

Weigh 1 g of pills, vitamin mix, premix or cut open capsule into a 500 ml screw glass jar, add about 400 ml redist. or deionized water, shake well and adjust to pH 4 - 5. Extract for 30 minutes at 95 °C (203 °F) in a water bath. During extraction the glass jar has to be shaken well at least five times. Chill down quickly to below 30 °C (86 °F), transfer the sample solution with redist. or deionized water quantitatively into a 1000 ml volumetric flask and fill up to the mark with redist. or deionized water. Transfer 1 ml into a 50 ml sterile centrifuge vial and fill up exactly to 40 ml with redist. or deionized water. Thereafter shake, sterile filter (or heat the sample 30 min at 95 °C in a water bath, thereafter chill down quickly below 30 °C (86 °F)) and, depending on the concentration range, further dilute in 1.5 ml (or 2.0 ml) sterile reaction vials with sterile water from the test kit.
Attention: For the calculation of the result, the predilution factor of 1000 has to be considered (1 g up to 1000 ml). The dilution step 1 ml up to 40 ml is already included in the standard curve.

6.3.2 Sample preparation with 0.2 sample size

Weigh 0.2 g of pills, vitamin mix, premix or cut open capsule (take weight into consideration) into a 50 ml centrifuge vial, add about 20 ml redist. or deionized water, shake well, adjust to pH 4 – 5 and fill up to 40 ml with redist. or deionized water. Extract for 30 minutes at 95 °C (203 °F) in a water bath. During extraction the vial has to be shaken well at least five times. It is important to make sure that the centrifuge vial is tightly closed. Chill down quickly to below 30 °C (86 °F). Thereafter centrifuge and, depending on the concentration range, further dilute the clear supernatant in 1.5 ml (or 2.0 ml) sterile reaction vials with sterile water from the test kit.

Note: For the evaluation the weight of sample has to be considered.

6.4. Added vitamin B1 in cereals, baby food, bread, flour and meat products

Weigh 1 g (ml) homogenized sample into a 50 ml sterile centrifuge vial, fill up to 30 ml with 0.1 mol / l HCl and shake well. Extract for 30 minutes at 95 °C (203 °F) in a water bath. During extraction the vial has to be shaken well at least five times. It is important to make sure that the centrifuge vials are tightly closed. Chill down quickly to below 30 °C (86 °F) and adjust to pH 4 to 5 with 2 mol / l NaOH. Fill up exactly to 40 ml with redist. or deionized water, shake, sterile filter (or heat the sample 30 min at 95 °C in a water bath. Thereafter chill down quickly below 30 °C (86 °F)) and, depending on the concentration range, further dilute in 1.5 ml (or 2.0 ml) sterile reaction vials with sterile water from the test kit.

6.5. Total vitamin content (native and added vitamin B1) in milk products, cereals and baby food

To extract the bound, native vitamin B1 as well or to determine it in non-fortified samples, the sample has to be extracted with enzyme. Procedures with additional treatments using enzymes are described in the literature. The following extraction procedure has proved itself:
Weigh 1 g (ml) homogenized sample into a 50 ml sterile centrifuge vial, add 20 ml redist. or deionized water, shake and adjust pH 4.5 with HCl.

Alternatively instead of water a citrate buffer can be used for the extraction (no pH adjustment is necessary); to 1 g sample add 20 ml citrate buffer pH 4.5 and shake.

Add 300 mg taka diastase, shake well and incubate 1 h in the dark at 37 °C (98.6 °F) (incubator or water bath, shake at times). Fill up exactly to 40 ml with redist. or deionized water. Thereafter, heat the extract for 30 min in a water bath at 95 °C (203 °F). During extraction the vial has to be shaken well at least five times. It is important to make sure that the centrifuge vial is tightly closed. Chill down quickly to below 30 °C (86 °F). Thereafter centrifuge and, depending on the concentration range, further dilute the clear supernatant in 1.5 ml (or 2.0 ml) sterile reaction vials with sterile water from the test kit.

6.6. Total vitamin content (native and added vitamin B1) in yeasts and yeast products

Weigh 1 g (ml) homogenized sample into a 50 ml sterile centrifuge vial, add about 10 ml H₂SO₄ 0.1 mol / l, shake well and autoclave 30 min at 121 °C (249.8 °F) (do not close the screw cap totally). Chill down quickly to below 30 °C (86 °F), add 3 ml of 2.5 mol / l sodium acetate solution and shake. Add to the sample solution 300 mg taka diastase and 10 mg acid potato phosphatase and shake again. Incubate over night (12 - 16 hours). Thereafter fill up to the mark 40 ml with dest. or redist. water. Extract for 30 minutes at 95 °C (203 °F) in a water bath. During extraction the vial has to be shaken well at least five times. It is important to make sure that the centrifuge vial is tightly closed. Chill down quickly to below 30 °C (86 °F). Thereafter centrifuge and, depending on the concentration range, further dilute the clear supernatant in 1.5 ml (or 2.0 ml) sterile reaction vials with sterile water from the test kit.
7. Test implementation

7.1. Test preparation

The **bottle with sterile water**: push the coloured lid up, pull off right up to the glass rim and turn entire lid to remove it. Each vitamin B1 standard is sufficient for 3 wells. Standards should be prepared freshly.

- open the vitamin B1 standard bottle, place the lid down with the opening facing upwards
- add \( x \) ml (\( x = \) see quality assurance certificate and label standard bottle) sterile water (from the test kit) to the standard bottle. Close the bottle with the lid and dissolve the standard by shaking = **standard concentrate**
- take 6 sterile vials (1.5 or 2.0 ml) and prepare from the dissolved standard concentrate a **standard curve** according to the following scheme:

<table>
<thead>
<tr>
<th>standard curve*</th>
<th>sterile water</th>
<th>standard concentrate</th>
<th>total volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>in mg / 100 g (ml)</td>
<td>in µl</td>
<td>in µl</td>
<td>in µl</td>
</tr>
<tr>
<td>blank: 0</td>
<td>850</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>standard 1: 0.012</td>
<td>850</td>
<td>+</td>
<td>150</td>
</tr>
<tr>
<td>standard 2: 0.024</td>
<td>700</td>
<td>+</td>
<td>300</td>
</tr>
<tr>
<td>standard 3: 0.036</td>
<td>370</td>
<td>+</td>
<td>300</td>
</tr>
<tr>
<td>standard 4: 0.048</td>
<td>200</td>
<td>+</td>
<td>300</td>
</tr>
<tr>
<td>standard 5: 0.060</td>
<td>200</td>
<td>+</td>
<td>600</td>
</tr>
</tbody>
</table>

*the sample extraction dilution of 1 : 40 is already included in the standard curve*

The **vitamin B1 assay - medium** is sufficient for 6 microtiter strips. Open the assay - medium bottle and remove the desiccant using tweezers (discard the desiccant).

- add 10 ml sterile water from the test kit to the vitamin B1 assay - medium bottle
- close the assay - medium bottle carefully and shake well
- heat the bottle in a water bath to 95 °C (203 °F) for 5 min while shaking at least twice; always make sure that the bottle is tightly closed
- quickly chill down to room temperature below 30 °C (86 °F)
- filter the medium through a 0.2 µm filter into a sterile 15 ml centrifuge vial
7.2. Test procedure

Only sterile samples which are diluted with sterile water from the test kit should be pipetted onto the microtiter plate.

– remove the **required strips** of the microtiter plate and place them into the additional holder. Return the unused strips together with the desiccant to the foil bag and seal it well, store at 2 - 8 °C (35.6 - 46.4 °F)

**Pipette first the assay – medium and then standard or diluted samples, as followed:**

– pipette 150 µl vitamin B1 assay - medium into the wells
– pipette 150 µl standard or diluted sample into the assigned wells (flush the pipette tip with standard or sample solution)
– cover the strips/cavities with adhesive foil: pull off the protective layer of the foil, place the foil flat onto the strips, smoothing it down by hand, press the foil firmly onto the strips
  **important:** make sure the cavities are sealed airtight by smoothing down the foil over the cavities, take special care with the wells around the edges
– incubate at **37 °C (98.6 °F)** in the dark for 44 - 48 h in an incubator

7.3. Measurement

– press down the adhesive foil once more, place the microtiter plate upside down on a table and dissolve the microorganisms thoroughly by shaking the plate on the surface of the desk
– invert the plate to the regular position and remove the adhesive foil diagonally, 180 degrees backwards, starting from the upper right; **the foil is strongly adhesive, so pulling it off the microtiter plate must be done with great care:** hold the strips firmly in the frame with one hand while you pull off the foil diagonally from the top right to the back
– destroy any bubbles on the surface of liquid in the wells (by means of a pipette tip or a needle)
– measure the turbidity with a microtiter plate reader at 610 - 630 nm (alternatively at 540 - 550 nm)
Note:
– after 44 - 48 h of incubation, the microtiter plate can be stored for max. 48 h in the refrigerator, thereafter the turbidity should be measured
– to avoid any time losses due to weekends or bank holidays, the microtiter plate can be evaluated after 60 h. It is recommended to use a timer to turn off the incubator after 44 - 48 h

8. Evaluation

A 4 - parameter evaluation is recommended, e. g. the RIDA® SOFT Win from R-Biopharm.

The test evaluation is correct on condition that
– OD blank < OD standard 1
– OD standard 5 > 0.6 OD

The sample dilution factor of 40 is already included in the standard curve (see Quality Assurance Certificate). In the below formula merely the further dilution factor of the extract and a differing sample weight need to be taken into consideration.

The calibration is carried out with thiamine hydrochloride. For the calculation of vitamin active thiamine (vitamin B1) the factor of 0.787 must be considered.

\[
\text{vitamin B1} = \frac{\text{conc. standard curve} \times \text{dilution factor} \times 0.787}{\text{sample weight} \text{ in g (ml)}
\]

Example:

Sample weight: 1 g
Sample extraction dilution: 1 : 40 (must not be considered)
Dilution of the sample extraction: 1 : 70 (has to be considered)
Measured concentration from the standard curve: 0.055 mg thiamine hydrochloride / 100 g (ml)

\[
0.055 \times 70 \times 0.787 / 1 = 3.03 \text{ mg active vitamin B1 (thiamine) / 100 g (ml)}
\]
Evaluation for capsules, pills, vitamin mixes:

vitamin B1 in mg/ pill or capsule =

\[
\text{conc. standard curve \times dilution factor \times pill or capsule weight in g \times 0.787} \\
\text{sample weight in g \times 100}
\]
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9. Literature

–Official Methods of Analysis (AOAC) 960.46
–Vitamin-Bestimmungen, Verlag Chemie GmbH Weinheim/Bergstr.; Strohecker und Henning