ID-Vit® Biotin Assay Kit

Microbiological test kit for the determination of total free biotin (Vitamin H) in serum using a Lactobacillus plantarum coated microtitre plate

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1. INTENDED USE

ID-Vit® Biotin Assay kit is a microtiter plate test kit based on a microbiological assay which measures the total free biotin 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 biotin content. For use in human and veterinary medicine and in research. The Biotin Assay kit is For Research Use Only.

2. INTRODUCTION

In the late 1950s the discovery of covalently bound biotin with a coenzyme function was reported. Biotin is a growth factor present in minute amounts in every living cell. The fact that humans have a requirement for biotin has been most clearly shown in 2 situations that result in biotin deficiency: 1) prolonged consumption of raw egg whites and 2) parenteral nutrition without biotin supplementation in patients with short-gut syndrome. Biotin plays an indispensable role in numerous naturally occurring carboxylation reactions. The daily biotin requirements for adults lie between 100 and 200 mg. Renewed interest in the role of biotin in human nutrition and therapy stems from the accumulating reports of various biotin-responsive syndromes.

Indications for a determination of biotin

- Defects in enzymes (e.g. genetic deficiency of biotinidase)
- Short gut syndrome
- Malnutrition

3. PRINCIPLE OF THE TEST

Serum samples are diluted and added into the microtiter plate wells coated with Lactobacillus plantarum which metabolizes biotin. The presence of biotin both in standards [STD] and samples gives a biotin-dependent growth response. After incubation at 37°C for 48 h, the growth of Lactobacillus plantarum is measured turbidimetrically at 610 - 630 nm (alternative at 540 - 550 nm) in an ELISA-reader. A dose response curve of absorbance unit (optical density, OD at 610 nm) vs. concentration is generated using the values obtained from standard. Biotin present in the patient samples is determined directly from this curve.
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>KIF007MTP</td>
<td>PLATE</td>
<td>One <em>Lactobacillus plantarum</em>-precoated microtiter plate, ready to use</td>
<td>12 x 8 wells</td>
</tr>
<tr>
<td>KIF007DI</td>
<td>DIL</td>
<td>Water 30 ml</td>
<td>4 x</td>
</tr>
<tr>
<td>KIF007ME</td>
<td>ASYMED</td>
<td>Biotin assay medium</td>
<td>4 x</td>
</tr>
<tr>
<td>KIF007ST</td>
<td>STD</td>
<td>Biotin Standard</td>
<td>4 x</td>
</tr>
<tr>
<td>KIF007FO</td>
<td>FOL</td>
<td>Cover plastic foil</td>
<td>4 x</td>
</tr>
<tr>
<td>KIF007FR</td>
<td>FRA</td>
<td>Replacement holder for 96-well plates</td>
<td>1 x</td>
</tr>
<tr>
<td>KIF007KO1</td>
<td>CTRL1</td>
<td>Biotin Control 1</td>
<td>4 x</td>
</tr>
<tr>
<td>KIF007KO2</td>
<td>CTRL2</td>
<td>Biotin Control 2</td>
<td>4 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 polyethersulfon filter with a sterile tip
- 15 ml centrifugal tubes, sterile (e.g. Falcon tubes)
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, controls, medium) in the test kit and store at 2-8°C.
- Take as many microtiter strips as needed from kit. Store unused strips in the original package bag 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 3 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).
- Water quality is extremely important. Only the water delivered with the test kit should be used for medium dilution, standard and control [CTRL1, CTRL2] reconstitution as well as for sample preparation.
- For sterile filtration, only a sterile polyethersulfone filter must be used.
- It is essential to run a standard curve for each separate assay.
- It is recommended to run a duplicate standard 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.
- By finishing the test, the used microtiter plates [PLATE] should be autoclaved.
- 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 3 days at 2-8°C in the dark. Biotin itself can be stored for longer at 2 - 8°C, but not the serum. Therefore, samples should be frozen at -20°C for longer storage.
- 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.
- Samples with visible amounts of precipitates should be centrifuged (5 min at 10000 g) prior to measurement and the resulting supernatant should be used in the test.

8.1 Sample dilution

Serum samples and controls [CTRL1, CTRL2] should be diluted 1:20 (= dilution factor) with water [DIL] from the kit prior to analysis:

50 µl sample + 950 µl water [DIL]

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. Put unused strips in the original package bag, and return the remaining parts of the test kit to the refrigerator. Bring all necessary reagents to room temperature.

Water [DIL] for medium [ASYMED], standard [STD], controls [CTRL1, CTRL2] and sample. 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 the test.
- Take the dry bag out of medium vial [ASYMED] by tweezers, shake off and discard.
- Add 10 ml of water to the assay medium, securely close the bottle and shake well. The amount is sufficient for 6 strips.
- Heat the bottle with medium in a water-bath at 95 °C for 5 min, while shaking well at least twice. It is important to make sure that the medium bottle is firmly closed at all times.
- Quickly cool the medium bottle to under 30 °C.
- Filter the medium sterilely with a 0.2 µm filter in a 15 ml centrifuge test tube.

**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:

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank: 0</td>
<td>500</td>
<td>0</td>
<td>= 500</td>
</tr>
<tr>
<td>Standard 1: 0,02</td>
<td>450</td>
<td>50</td>
<td>= 500</td>
</tr>
<tr>
<td>Standard 2: 0,06</td>
<td>350</td>
<td>150</td>
<td>= 500</td>
</tr>
<tr>
<td>Standard 3: 0,10</td>
<td>250</td>
<td>250</td>
<td>= 500</td>
</tr>
<tr>
<td>Standard 4: 0,14</td>
<td>150</td>
<td>350</td>
<td>= 500</td>
</tr>
<tr>
<td>Standard 5: 0,18</td>
<td>50</td>
<td>450</td>
<td>= 500</td>
</tr>
</tbody>
</table>
Controls [CTRL1, CTRL2]
- The controls must be freshly prepared before the test.
- Open the bottles of controls [CTRL1, CTRL2], remove seal. Dispose of screw-top lid and seal.
- Add 0.2 ml water [DIL] from the test kit to the control bottles [CTRL1, CTRL2], close the bottles and dissolve by vortexing the bottles (=control 1 and control 2).
- Treat the control afterwards as the sample is treated.
- Pipette 150 µl of the reconstituted controls [CTRL1, CTRL2] into each well. We recommend to run a duplicate.
- For the concentration of the controls [CTRL1, CTRL2] please see Control specification.

9.2. Test Initiation

- Take as many microtiter strips as needed from the kit in put them in the second microtiter strip holder [FRA]. Store unused strips in the original package bag at 2-8° C to prevent contamination or moisture exposure.
- A medium solution is sufficient for 6 strips.
- Put 150 µl biotin assay medium [ASYMED] in the cavities.
- Add 150 µl standard [STD], controls [CTRL1,CTRL2], respectively, sample in the cavities. Pre-rinse the pipette tip with standard, control and sample solution respectively.
- Carefully seal the cavities with plastic foil [FOL]. Important: the cavities must be made airtight by pressing down with the hand!
- Keep at 37 °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 over again and carefully remove the foil [FOL], beginning with the lower, left corner and pulling diagonally backwards at an angle of 180°.
- 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)

Please note
- After 48 hrs incubation time, the microtiter platter 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 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

Biotin in µg/l = Value from the standard curve x dilution factor

Reference value for human serum

Biotin (n = 84)
- < 750 ng/l biotin deficiency requiring treatment
- 750 – 1250 ng/l suboptimal biotin status
- >1250 ng/l sufficient levels of biotin

Please note: A concentration range of 0.4 - 3.6 µg/L biotin is covered at a sample dilution 1:20. We recommend each laboratory to develop its own normal range. The values mentioned above are only for orientation and can deviate from other published data.

11. PERFORMANCE CHARACTERISTICS (obtained using human serum)

Precision and reproducibility

<table>
<thead>
<tr>
<th>Intra-Assay (n=5)</th>
<th>Biotin [µg/l]</th>
<th>CV [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>63.5</td>
<td>3.4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Inter-Assay (n=5)</th>
<th>Biotin [µg/l]</th>
<th>CV [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>64.3</td>
<td>2.9</td>
</tr>
</tbody>
</table>
**Recovery**

Samples from 4 patients were diluted differently (20, 40, 80, 120, 160), spiked with Biotin and analyzed. The mean values are shown below:

\( n = 15 \)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean value measured in original sample [µg/L]</th>
<th>Spike [µg/L]</th>
<th>Biotin expected [µg/L]</th>
<th>Biotin measured [µg/L]</th>
<th>Recovery Rate [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1.1</td>
<td>2</td>
<td>3.1</td>
<td>3.3</td>
<td>110</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>5.1</td>
<td>5.2</td>
<td>103</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>7.1</td>
<td>7.3</td>
<td>103</td>
</tr>
</tbody>
</table>

Recovery rate in total [%] 105

\( n = 14 \)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean value measured in original sample [µg/L]</th>
<th>Spike [µg/L]</th>
<th>Biotin expected [µg/L]</th>
<th>Biotin measured [µg/L]</th>
<th>Recovery Rate [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>1.5</td>
<td>2</td>
<td>3.5</td>
<td>3.2</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>5.5</td>
<td>4.7</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>7.5</td>
<td>7.4</td>
<td>98</td>
</tr>
</tbody>
</table>

Recovery rate in total [%] 88
### Sample Measurement Results

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean value measured in original sample [µg/L]</th>
<th>Spike [µg/L]</th>
<th>Biotin expected [µg/L]</th>
<th>Biotin measured [µg/L]</th>
<th>Recovery Rate [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>1.4</td>
<td>2</td>
<td>3.4</td>
<td>3.3</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>5.4</td>
<td>5.6</td>
<td>105</td>
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<tr>
<td></td>
<td></td>
<td>6</td>
<td>7.4</td>
<td>7.0</td>
<td>93</td>
</tr>
</tbody>
</table>

**Recovery rate in total [%] 98**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean value measured in original sample [µg/L]</th>
<th>Spike [µg/L]</th>
<th>Biotin expected [µg/L]</th>
<th>Biotin measured [µg/L]</th>
<th>Recovery Rate [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>D</td>
<td>1.3</td>
<td>2</td>
<td>3.3</td>
<td>3.6</td>
<td>115</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>5.3</td>
<td>5.7</td>
<td>110</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>7.3</td>
<td>6.9</td>
<td>93</td>
</tr>
</tbody>
</table>

**Recovery rate in total [%] 106**
**Linearity**

Two patient samples were diluted and analyzed. The results are shown below: n = 2

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dilution</th>
<th>Expected [µg/L]</th>
<th>Measured [µg/L]</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>20</td>
<td>3.0</td>
<td>3.1</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td></td>
<td>3.5</td>
</tr>
<tr>
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<td>80</td>
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<td>3.3</td>
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<tr>
<td></td>
<td>120</td>
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<td>2.9</td>
</tr>
<tr>
<td></td>
<td>160</td>
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<td>3.4</td>
</tr>
<tr>
<td>C</td>
<td>20</td>
<td>1.3</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td></td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td></td>
<td>1.6</td>
</tr>
</tbody>
</table>

**12. REFERENCES**

13. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- Biotin Assay Test components contain organic solvents. Contact with skin or mucous membranes must be avoided.
- All reagents in the test package are for use in human and veterinary medicine and in research. For Research 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 Biotin Assay.

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For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.