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Vitamin H (Biotin) ELISA

Catalog Number M046019

For the quantitative determination of biotin in serum, plasma and urine samples.

For research use only.
This product insert must be read in its entirety before using this product.

INTENDED USE

The Biotin test is an enzyme binding assay in microtiter format for the quantitative detection of Biotin (vitamin H) in serum, plasma or urine.

SUMMARY AND EXPLANATION

Biotin, also known as vitamin H, is of great importance for the biochemistry of the human organism. As a prosthetic group of mitochondrial enzymes (carboylases), biotin plays a central role as a CO_2-carrier in important metabolic reactions such as gluconeogenesis, synthesis of fatty acids and metabolism of amino acids. Furthermore, biotin influences the growth and maintenance of blood cells, sebaceous glands, skin, hair and nails. Next to the free form of biotin, the biotin linked to lysin, also known as biocytine, can also be utilized as a vitamin source by the body, after cleavage from the protein by the enzyme biotinidase.

In nature, biotin is very common. It can be found in bacteria and mushrooms, as well as in higher plant life and animal tissue—especially in liver and kidney. However, the biotin availability in some food is very slim. Amounts worth mentioning can only be found in yeast, soybeans, nuts, cauliflower, lentils, oats, wheat germ and in egg yolk whereas fruits, milk products and most vegetables only contain small amounts. Solely, the amount of biotin is not the decisive factor, but rather the bioavailability. The bioavailability varies quite strongly and depends on the kind of food and also on the extent to which biotin is protein-bound. For example, whereas in plants, biotin is available in the free form, food coming from animals contains mostly protein-bound biotin. Only after proteolytic reduction in the small intestine followed by a cleavage with intestine (pancreas)-biotinidase is the biotin available in the free absorbable form.

Biotin which is synthesized endogenously by flora of the intestine is not reabsorbed in the colon, but it is stored as protein-bound biotin in the bacteria of the intestine and is thus not available to cover the biotin requirement of the organism. The treatment of food also causes losses in biotin. Wheat, as a whole grain, contains 4 times as much biotin as all-purpose flour (Type 405). The alimentary utilization of biotin is estimated at 50%, so that a biotin deficiency can easily result. Malnutrition as well as an inherited disorder in biotin metabolism (singular or multiple deficiency in carboxylase, biotinidase deficiency) can lead to a biotin deficiency. Furthermore, circumstances of life, where an increased biotin demand exists (pregnancy, nursing, athletic activities, pathological conditions) may cause a biotin malnutrition. A variety of disorders on hair, skin and nails are the medically relevant consequences of a biotin deficiency. The symptoms range from brittle, splintered fingernails to different forms of alopecia to scaly erythematous and seborrheic dermatitis. Animals have also been observed with similar illnesses, ranging from larger skin detachments, epidermal crust development, as well as a hyper- and parakeratosical change in mucous membrane.

With an average nutrition, a daily ingested amount of 30–100 µg of biotin is considered sufficient by both the Deutsche Gesellschaft für Ernährung (DGE) and the American Recommended Dietary Allowances (RDA).

The normal biotin plasma level ranges from 200–1200 ng/L. An optimum plasma concentration of biotin in healthy humans is considered to be 400 ng/L. Since these values can differ by 100% from one day to the next, it is advised that a biotin detection must be carried out on 2–3 consecutive days to ensure a correct diagnosis of the deficiency as well as to observe the development of a substitution therapy.

Independent from the cause, a biotin deficiency always exists when the plasma biotin level is below 100 ng/L. In this case, substitution should definitely occur.

If biotin is taken externally, excessive amounts are not absorbed and excreted with the feces. By contrast, absorbed amounts of biotin which exceed the storage capacity of the organism are eliminated in the urine. Shortly after an oral take up of biotin, the biotin concentration in the plasma increases several times, but after 24 h the average value is reached again. In pharmacological dosages (mg-range), biotin stimulates the differentiation of epidermal cells. The effect is independent from the biotin status and influences all keratin structures, like hair, skin and nails.

Recent studies have shown that the elderly with 300 ng/L have a lower biotin level than younger adults and children, whose optimal range lies between 400 and 500 ng/L. The biotin concentration in urine of all age groups is approximately 30 to 40 times higher than the respective serum concentration.

The quantitative detection of biotin in serum, plasma and urine is easier and faster to carry out with the ELISA than with the common microbiological procedures and isotope dilution tests. The samples can be applied directly to the test after required dilution.

PRINCIPLE OF THE ASSAY

The basis of the test is the extremely high affinity of avidin (a protein produced by microorganisms) to biotin. The microtiter plates are coated with avidin. The enzyme-labeled biotin (Conjugate) and the sample or the Biotin Standard Solution are added. Free and enzyme-labeled biotin compete for the avidin-binding sites. Any unbound, enzyme-labeled biotin is then removed by a washing step. The detection occurs with the addition of Substrate (urea peroxide) and Chromogen (tetramethylbenzidine). The Conjugate, that is bound to avidin, changes the colorless Chromogen to a blue end product. The addition of the Stop Solution causes a color change from blue to yellow. The measurement is done photometrically at 450 nm (optional: reference wavelength ≥ 600 nm). The resulting absorbance values are inversely proportional to the biotin concentration of the sample.

KIT COMPONENTS

Microtiter Plate - The plate contains 12 x 8-well strips coated with avidin. Ready for use.

Standard 1 - 1 vial aqueous solution without biotin, contains 0.01% Thimerosal. Ready to use.

Standard 2 - 1 vial of a 37 ng/L biotin aqueous solution, contains 0.01% Thimerosal. Ready to use.

Standard 3 - 1 vial of a 111 ng/L biotin aqueous solution, contains 0.01% Thimerosal. Ready to use.

Standard 4 - 1 vial of a 333 ng/L biotin aqueous solution, contains 0.01% Thimerosal. Ready to use.

Standard 5 - 1 vial of a 1000 ng/L biotin aqueous solution, contains 0.01% Thimerosal. Ready to use.

Control Serum A and B - 1 vial diluted human serum, ready to use. For biotin concentration see kit enclosure (Note): Contains < 0.1% Kathon CG and < 0.1% Tween 20.

Dilution Buffer - 1 vial of a buffered solution containing 0.01% Thimerosal, pH 7.4. Ready to use.

Conjugate Concentrate (Red Cap) - 1 vial of 11-fold concentrated biotinylated peroxidase.

Wash Buffer - 1 vial of PBS salt, pH 7.4, contains Tween 20.

Substrate (Green Cap) - 1 vial of Substrate containing urea peroxide. Ready to use.

Chromogen (Blue Cap) - 1 vial of tetramethylbenzidine (TMB). Protect from light.

Stop Solution (Yellow Cap) - 1 vial of 1 M Sulfuric Acid. Ready to use.

STORAGE

Unopened Kit	Store at 2–8º C. Do not use past the kit expiration date.	
Opened Reagents	Standards	Store at 2–8º C.
	Dilution Buffer	
	Serum Control	
	Conjugate	
	Substrate	
	Chromogen	
	Stop	
	Wash Buffer	
	Microtiter wells	Return unused wells to the foil pouch containing the desiccant and seal. Store at 2–8º C.

SUPPLIES REQUIRED BUT NOT PROVIDED

- Microplate Reader
- Microplate Washer
- Pipettes or pipetting equipment with disposable polypropylene tips (10 µL, 100 µL, 1 mL)
- Multi-channel pipette
- Disposable polypropylene test tubes
- Glass measuring cylinders
- Distilled or deionised water

PRECAUTIONS

- The Standards as well as the Dilution Buffer contain 0.01% Thimerosal. Contact with skin or mucous membranes must be avoided.
- The Washing Buffer contains Tween 20. It is irritating to eyes, respiratory system and skin.
- Urea peroxide can cause cauterization. Handle with care!
- The Stop Solution contains 1 M sulfuric acid. Avoid contact with skin and clothing! Patient samples should be considered potentially contagious and be treated with the necessary safety precautions.
- All reagents and materials coming in contact with potential infectious specimens must be treated with disinfectants or autoclaved at 121 °C for at least one hour.
- An exchange of reagents between kits of different lot numbers is not possible.

CRITICAL PARAMETERS

- Allow samples and all reagents to equilibrate to room temperature (22–25 °C) prior to performing the assay. This is especially important for the TMB Substrate.
- Adhere to recommended incubation temperatures in the protocol as variations may cause inconsistent or poor assay results.
- It is essential that all wells are washed thoroughly and uniformly. When washing is done by hand, use a squeeze bottle and ensure that all wells are completely filled and emptied at each step.
- Use only reagents from the same lot for each assay. This is especially important when running more than one plate per sample group.
- A separate standard curve must be run on each plate.
- Reagents can easily be trapped in the caps of small microfuge tubes. It is recommended to briefly centrifuge these reagents before use.
- Mix all reagents thoroughly prior to use, but avoid foaming!
- Keep the wells sealed with the plate sealer except when adding reagents and during reading.
- Any variation in the protocol can cause variation in binding!
- The kit should not be used beyond the expiration date on the kit label.
- The values obtained by the samples should be within the standard range. If this is not the case, dilute the sample and repeat the assay.
- We take great care to ensure that this product is suitable for all validated sample types, as designated in this manual. Other sample types may be tested and validated by the user.

SAMPLE COLLECTION AND STORAGE

This test is suitable for serum, plasma and urine samples. The test should be carried out with freshly taken samples. Samples can be stored at 2–8 °C for 48 h. For longer storage, serum and urine samples should be frozen at -20 °C.

REAGENT PREPARATION

Note: All reagents should be stored at the recommended temperatures. Bring all reagents to room temperature (22 - 25 °C) before use. Reagents can easily be trapped in the caps of small microfuge tubes. It is recommended to briefly centrifuge these reagents before use.

Wash Buffer - The whole contents of the pouch with salt for the Washing Buffer is dissolved in 1000 mL distilled or deionized water. The Buffer can be used four weeks if stored at 2–8 °C.

Conjugate - The Conjugate (vial with red cap) comes in a concentrated form. Since the diluted Conjugate has a limited stability, only the amount which actually is needed should be reconstituted. Before pipetting, the Conjugate should be shaken carefully. The Conjugate is diluted 1:11 with the Dilution Buffer (e.g. 200 µL Conjugate + 2.0 mL Dilution Buffer, sufficient for 4 Microtiterstrips).

SAMPLE PREPARATION

Serum and plasma samples require a 1:3 dilution prior to assay (e.g. 100 µL serum + 200 µL Buffer). If possible, they should be clear and free of bacterial contamination.

Urine samples require a 1:21 dilution prior to assay (e.g. 100 µL urine + 2.0 mL Buffer).

ASSAY PROTOCOL

Read the entire protocol before beginning the assay. It is recommended that all standards and samples be assayed in duplicate. Reagents can easily be trapped in the caps of small microfuge tubes. It is recommended to briefly centrifuge these reagents before use. *Note: Reagents and samples may require specific handling temperatures and need preparation prior to the assay. See the Reagent and Sample Preparation sections before proceeding. Note: The control serum is adjusted to a lot specific value. It must not be used with test kits from other lots. Standards and control serum are ready for use and must not be diluted.*

1. Prepare all reagents and samples as described in the previous sections.
2. Remove any excess microtiter strips from the plate frame and return them to the foil pouch containing the desiccant pack.

Standard/Sample Incubation

3. Pipet 50 µL of standard, serum control, or diluted sample into duplicate wells.
4. Incubate for 30 minutes at room temperature (22–25 °C).

Conjugate Incubation

5. Pipet 50 µL of diluted conjugate to each well.
6. Incubate for 30 minutes at room temperature.

Wash

7. Aspirate and wash each well four (4) times with the Working Wash Solution, using an automatic microplate washer. Blot dry by inverting the plate on an absorbent material. The wash solution volume should be set to dispense 250 µL into each well.

Substrate Incubation

8. Pipet 50 µL of the Substrate into each of the wells.
9. Pipet 50 µL of the Chromogen into each of the wells. Mix reagents by gently tapping the side of the plate.
10. Incubate in the dark for 30 minutes at room temperature.

Stop Reaction

11. Add 100 µL of the Stop Solution into each of the wells. Mix gently.
12. Read the absorbance of the solution in the wells within 60 minutes, using a microplate reader set to 450 nm (optional reference filter $\geq$ 600 nm) against an air blank.

SUMMARY

Prepare reagents and samples as previously described.

⇩

Pipet 50 µL Standard, serum control or diluted sample in duplicate into the wells.
Incubate for 30 minutes at room temperature (22 - 25 °C).

⇩

Pipet 50 µL diluted Conjugate into the wells.
Incubate for 30 minutes at room temperature.

⇩

Aspirate and wash 4 times.

⇩

Add 50 µL Substrate to each well.

⇩

Add 50 µL Chromogen to each well.
Incubate in the dark for 30 minutes at room temperature.

⇩

Add 100 µL of Stop Solution to each well. Read at 450 nm.

PROCEDURAL NOTES

- Since the biotin level of an individual differs by 100% on a daily basis, it is advised that the determination should be carried out over a 2–3 day period to ensure a correct diagnosis of biotin deficiency. This procedure should also be followed as a development control during a biotin therapy (oral substitution therapy). The samples should always be taken under equal conditions (on an empty stomach).

  critical values:
  ≤ 100 ng/L .... biotin deficiency requires treatment
  100 - 250 ng/L .... suboptimal biotin supply
  ≥ 250 ng/L .... sufficient biotin supply

- Furthermore, in pharmacological dosages (mg-range), biotin stimulates the differentiation of epidermal cells. The effect is independent from the biotin status and influences all keratin structures, like hair, skin and nails.

CALCULATION OF RESULTS

The mean values of the absorbance values obtained for the standards and the samples are divided by the absorbance value for Standard 1 (zero standard) and multiplied by 100. The zero standard is thus made equal to 100%, and the absorbance values are quoted in percentages.

$$\% \text{ absorbance} = \frac{100 \times \text{absorbance Standard (or sample)}}{\text{absorbance zero standard}}$$

The calculated %-values for the standards are transferred into a system of coordinates on semilogarithmic graph paper against the known concentrations of the Biotin Standards (ng/L). The calibration curve should be virtually linear in the range of Standards 3–6. The biotin concentration (ng/L) of the diluted patient samples can be read from the calibration curve. After that, multiplication by the dilution factor 3 gives the biotin contents of the serum.

CLINICAL RESULTS

Plasma biotin concentrations depending on the age.
In a study at the Friedrich-Schiller Universität Jena it could be shown that depending on the age, different biotin levels can be measured. In this study, the average biotin concentration was higher than 400 ng/L in the plasma of children and young adults. In contrast, the measured concentration in the elderly was significant lower with 270 ng/L.

	Biotin concentration (ng/L) in plasma
Children n = 20; 13–14 years	420 ± 230
Adults n = 35; 21–35 years	460 ± 380
Elderly n = 33; 63–86 years	270 ± 100

Cross-Reactivity

The cross-reactivity of the Biotin test was investigated with structurally similar substances. With exception of biocytin (protein-bound biotin), there was no significant cross-reactivity with other substances similar to biotin.

Substance	% Cross-reactivity
biotin	100
biocytin	83
biotin-d-sulfoxid	34.7
biotin-l-sulfoxid*	24.5
biotinsulfon	28.2
bisnorbiotin	5.5

**biotin-l-sulfoxide is contaminated with 3.3% biotin and not a quantified amount of biotin-d-sulfoxid.*

Reproducibility

Intra-assay Precision (Precision within an assay) - The intra-assay precision was measured by assaying three control samples 5 times on one plate.
Inter-assay Precision (Precision between assays) - The inter-assay precision was assessed by repeated measurements of three control samples in 24 different assays.

	Intra-assay Precision			**Inter-assay Precision**		
Control	1	2	3	1	2	3
OD	0.181	1.215	1.439	0.193	1.181	1.409
CV (%)	8.8	7.3	7.5	6.4	5.7	5.6

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