Version 10-04/24

User's Manual

Biotin ELISA Kit

REF **DEIA281**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

Enzyme Immunoassay for the Quantitative Determination of Biotin (Vitamin H) in Food.

General Description

Biotin serves as the prosthetic group of enzymes, which catalyze carboxylations in the organism. For this purpose, biotin is bound via its carboxy group to lysin residues of carboxylases, and the transfer of carbon dioxide takes place after its attachment to a nitrogen atom of biotin, forming the so-called active carbon dioxide.

The awareness of the population for a good health and its interest in healthy nutrition has increased significantly during the last years. After the content of vitamins in his nourishment has gained importance for the consumer, food has partially been vitaminized by the manufacturer.

When there exists a lack of biotin, seborrhoea, dermatitis, anorexia, muscle pain, tiredness and nervous disorders can appear. As biotin is synthesized by the human intestinal flora, deficiency symptoms are rare, appear however after excessive ingestion of raw egg white, which can be explained by its content of biotinbinding avidin.

Principles of Testing

The Biotin (Vitamin H) quantitative test is based on the principle of the enzyme linked immunosorbent assay. Avidin, which shows a high affinity to biotin, is bound on the surface of a microtiter plate. Biotin containing samples or standards and a biotin-alkaline phosphatase conjugate are given into the wells of the microtiter plate. Enzyme labeled and free biotin compete for the 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 30 minutes, resulting in the development of a yellow colour. The colour development is inhibited by the addition of a stop solution. The yellow colour is measured photometrically at 405 nm. The concentration of biotin is indirectly proportional to the colour intensity of the test sample.

Reagents And Materials Provided

The kit contains reagents for 96 determinations. They have to be stored at 2-8°C. Expiry data are found on the labels of the bottles and the outer package.

1. Microtiter plate consisting of 12 strips with 8 breakable wells each, coated with avidin.
2. Biotin Standards (0; 1; 2.5; 5; 10; 25 ng/mL): 6 vials with 0.5 mL each, ready-to-use. If stored in a refrigerator, crystals could precipitate, which can be redissolved by a 15 minutes incubation in a water bath to 37°C.
3. Conjugate (Biotin-Alkaline Phophatase): 15 mL, contains 0.1% sodium azide, dyed red, ready-touse.
4. Substrate Solution (PNPP): 15 mL; ready-to-use.
5. Stop Solution (1 M NaOH): 15 mL; ready-to-use.
6. Standard/Sample Diluent (PBS): 2 × 50 mL, contains 0.1% sodium azide, ready-to-use. If stored in a

refrigerator, crystals could precipitate, which can be redissolved by 15 minutes incubation in a water bath to 37°C.

7. Washing Solution (PBS + Tween 20): 30 mL as 10× concentrate, dyed blue. Dilute 1+9 with distilled water. If during the cold storage crystals precipitate, the concentrate should be warmed up to 37°C for 15 minutes.
8. Two plastic foils to cover the strips during the incubation.
9. Plastic bag to store unused microtiter strips.
10. Instruction Manual.

Materials Required But Not Supplied

1. 50, 100 and 1000 µL-micropipets
2. Volumetric flask
3. Mortar, mixer
4. Centrifuge
5. ELISA reader (405 nm)
6. Potassiumhexacyanoferrate(II)-3-hydrate (150 g/L; CarrezI)
7. Zincsulfate-7-hydrate (300 g/L; Carrez II)
8. Double-distilled water
9. 1 M caustic soda solution
10. 1 M hydrochloric acid

Storage

Store kit at 2-8°C. It is stable up to the expiry date stated on the label of the box. Do not use kit beyond its expiry date.

Specimen Collection And Preparation

The vitamin is extracted from the sample by doubledistilled 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. The sample solutions must be diluted such, that the concentrations lie within the linear range of the calibration curve.

1. Multivitamin Tablets and Capsules

Two(2) grams of crushed tablets or capsules are dissolved in 50 mL 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 100 mL by double-distilled water. The solid matter is separated by centrifugation, and 100 µL of the upper phase is diluted with 900 µL of sample diluent (total dilution factor 500) and used in the test. To dissolve the capsules, heating to 30-40°C is recommended.

2. Multivitamin and Orange Juices

Ten(10) mL juice is diluted in a beaker with 20 mL of double- distilled water and adjusted to pH 6-7. 0.5 mL each of Carrez I and Carrez II are added, and the solution is filled up to 50 mL by double-distilled water. The solid matter is separated by centrifugation, and 100 µL of the upper phase is diluted with 900 µL of sample diluent (total dilution factor 50) and used in the test.

3. Multivitamin Jam

Five(5) grams of marmalade are weighed into a beaker and homogenised with 30 mL of double-distilled water in a mixer. 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 50 mL with double-distilled water. The solid matter is separated by centrifugation, and 100 µL of the upper phase is diluted with 900 µL of sample diluent (total dilution factor 100) and used in the test.

4. Grain Products (Corn Flakes and Muesli)

Five(5) grams of sample are homogenised by a mortar or a mixer and extracted by 50 mL of double-distilled water. After centrifugation, 20 mL of the supernatant is adjusted to pH 6-7, and 0.5 mL each of Carrez I and Carrez II are added. Afterwards the solution is filled up to 25 mL by double-distilled water. The solid matter is separated by centrifugation, and 100 µL of the upper phase is diluted with 900 µL of sample diluents (total dilution factor 100) and used in the test.

5. Multivitamin Sweets

Two(2) grams of crushed sweets are dissolved by gentle heating (if necessary) in 50 mL of 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 100 mL with double-distilled water. The solid matter is separated by centrifugation, and 50 µL of the upper phase is diluted with 950 µL of sample diluent (total dilution factor 1000) and used in the test.

6. Isotonic Powder

Two(2) grams of isotonic powder are dissolved in 50 mL of doubledistilled 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 100 mL with double-distilled water. The solid matter is separated by centrifugation, and 100 µL of the upper phase is diluted with 900 µL of sample diluent (total dilution factor 500) and used in the test.

7. Milk

Five(5) milliliters 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.

8. Dry Milk Instant Formula

Ten(10) grams 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.

Assay Procedure

1. Prepare samples as described above.

2. Pipet 50 µL standards or prepared samples in duplicate into the appropriate wells of the microtiter plate. Immediately add 100 µL biotin-AP conjugate into each well.
3. Cover the microtiter plate with a plastic foil and incubate for 60 minutes at room temperature on a microtiter plate shaker (or 90 minutes without shaker).
4. Wash the plate three times as follows: Discard the contents of the wells (dump or aspirate). Pipet 300 µL of diluted washing solution into each well. After the third repetition empty the wells again and remove residual liquid by striking the plate against a paper towel. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbencies.
5. Pipet 100 µL of substrate solution into each well.
6. Allow the reaction to develop in the dark (e.g. cupboard or drawer; the chromogen is lightsensitive) for 30 minutes at room temperature.
7. Stop enzyme reaction by adding 100 µL of stop solution (1M NaOH) into each well. The yellow colour will darken upon addition.
8. After thorough mixing, measure absorbance at 405 nm (reference wavelength 620 nm), using an ELISA reader. The colour is stable for 30 minutes.

Calculation

1. Calculate the average optical density (OD 405 nm) for each set of reference standards or samples.
2. Construct a standard curve by plotting the mean optical density obtained for each reference standard against its concentration in ng/mL on semi-log graph paper with the optical density on the vertical (y) axis and the concentration on the horizontal (x) axis.
3. Using the mean optical density value for each sample, determine the corresponding concentration of biotin (Vitamin H) in ng/mL from the standard curve. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed.

The diluted samples must be further converted by the appropriate dilution factor. The factor is dependent on the sample preparation procedure employed. Applying the procedures for milk and dry milk instant formula the dilution factors are 5 or 25 respectively.

The following table contains an example for a typical standard curve. The binding is calculated as percent of the absorption of the 0 ng/mL standard. These values are only an example and should not be used instead of the standard curve which has to be measured in every new test.

Biotin (ng/mL)	(% binding of 0 ng/mL)
0	100
1.0	91
2.5	77
5.0	38
10	12
25	4

Precision

Intra-assay Precision

The intra-assay variation of the biotin test was determined to 3%.

Sensitivity

The sensitivity of the Biotin (Vitamin H) ELISA is 0.5 ng/mL (based on the standard curve).

Recovery

The recovery of spiked samples was determined to 98%.

Precautions

1. Prior to beginning the assay procedure, bring all reagents to room temperature (20-25°C).
2. All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
3. Once the assay has been started, all subsequent steps should be completed without interruption and within the recommended time limits.
4. Replace caps in all the reagents immediately after use. Do not interchange vial stoppers.
5. Use a separate disposable tip for each specimen to prevent cross-contamination.
6. All specimens and standards should be run at the same time, so that all conditions of testing are the same.
7. Do not mix components from different batches.
8. Do not use reagents after expiration date.
9. Check both precision and accuracy of the laboratory equipment used during the procedure (micropipets, ELISA reader etc.).
10. **HEALTH AND SAFETY INSTRUCTIONS**

1) Do not smoke or eat or drink or pipet by mouth in the laboratory.
2) Wear disposable gloves whenever handling patient specimens.
3) Avoid contact of substrate and stop solution with skin and mucosa (possible irritation, burn or toxicity hazard). In case of contact, rinse the affected zone with plenty of water.
4) Handling and disposal of chemical products must be done according to good laboratory practices (GLP).