Vitamin H (Biotin) ELISA Kit

Enzyme Immunoassay for the quantitative determination of Vitamin H (Biotin) in food

Catalog number: ARG80817

For research use only. Not for use in diagnostic procedures.

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MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: No. 22, Ln. 227, Gongyuan Rd., Hsinchu City 300, Taiwan

Phone: +886 (3) 562 1738

Fax: +886 (3) 561 3008

Email: info@arigobio.com

INTRODUCTION

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 sig-nificantly 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 biotin-binding avidin.

PRINCIPLE OF THE ASSAY

This assay employs the competitive quantitative enzyme immunoassay technique. Avidin is coated 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 antibody 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 color. The color development is inhibited by the addition of a stop solution. The yellow color is measured at 405 nm. The concentration of Biotin is indirectly proportional to the color intensity of the test sample.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Avidin-coated microplate	12 strips x 8-well	4°C
Biotin-Alkaline Phophatase Conjugate	15 ml (ready to use)	4°C
Standards (0,1, 2.5, 5, 10, 25 ng/ml)	6 X 0.5 ml (ready to use)	4°C
Sample dilution buffer	2 X 50 ml (ready to use)	4°C
10x Wash Buffer	30 ml	4°C
PNPP substrate	15 ml	4°C (Protect from light)
STOP solution	15 ml	4°C
Plastic foils	2	

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Briefly spin down the Biotin-Alkaline Phophatase conjugate before use.
- If crystals are observed in the 10X Wash buffer and Sample diluent buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
- Samples contain azide cannot be assayed.

SAMPLE COLLECTION & STORAGE INFORMATION

The vitamin is extracted from the sample by double-distilled 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) und 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.

Multivitamin Tablets and Capsules

The tablets and capsules are dissolved in 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 a defined volume by double-distilled water. The solid matter is separated by centrifugation, and the upper phase is further diluted by sample diluent. To dissolve the capsules, heating to 30-40°C is recommended.

Multivitamin Juices

The juice is adjusted to pH 6-7, 0.5 ml each of Carrez I and Carrez II are added, and the solution is filled up to a defined volume by double-distilled water. The solid matter is separated by centrifugation, and the upper phase is further diluted by sample diluent.

Multivitamin Jam

The jam is homogenised in a mixer, and approximately 8 grams are extracted by 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 a defined volume by double-distilled water. The solid matter is separated by centrifugation, and the upper phase is further diluted by sample diluent.

Grain Products (Corn Flakes and Muesli)

3-5 grams of sample are homogenised by a mortar or a mixer, extracted by 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 a defined volume by double-distilled water. The solid matter is separated by centrifugation, and the upper phase is further diluted by sample diluent. Grain products normally contain low concentrations of vitamin B12. In order to avoid high

dilutions, the sample can be extracted directly by sample diluent instead of double-distilled water.

Multivitamin Sweets

The sweets are dissolved by gentle heating (if necessary) in 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 a defined volume by double-distilled water. The solid matter is separated by centrifugation, and the upper phase is further diluted by sample diluent.

Dry Milk Instant Formula

10 g 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.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 10X wash buffer into distilled water to yield 1X wash buffer.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Pipet 50 µl standards or prepared samples in duplicate into the appropriate wells of the microtiter plate. Immediately add 100 µl biotin-

AP into each well.

3. Cover the microtiter plate with a plastic foil and incubate for 60 minutes at RT.
4. Aspirate each well and wash, repeating the process 2 times for a total 3 washes. Wash by filling each well with 1X wash buffer (350 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
5. Add 100 µl of substrate solution to each well. Incubate for 30 minutes at room temperature in dark.
6. Add 100 µl of Stop Solution to each well.
7. Read the OD with a microplate reader at 405 nm immediately.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

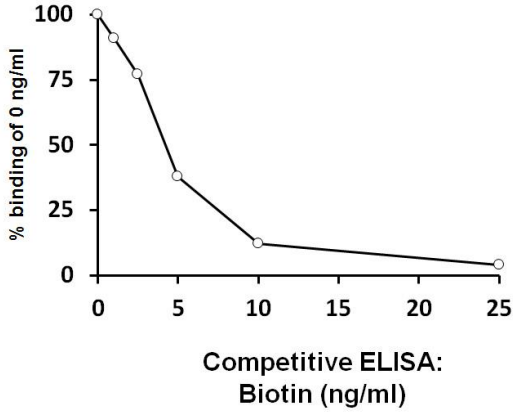
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter

Logistics is the preferred method. Other data reduction functions may give slightly different results. The diluted samples must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

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

Intra-assay

The CV value of intra-assay precision was 3%.

Recovery

98%