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Manual

IDK® Biotin ELISA

For the in vitro determination of biotin (vitamin H) in serum, plasma, urine and milk

Valid from 2019-11-13

REF **K 8141**

Σ 96

+8 °C
+2 °C

IVD

CE

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1. INTENDED USE

This Immundiagnostik AG assay is intended for the quantitative determination of biotin in serum, plasma, urine and milk. For *in vitro* diagnostic use only.

2. INTRODUCTION

Biotin (vitamin H) is present in bacteria, funghi, plants and animals. In food, the major part of biotin is covalently bound to protein, leaving only a minor fraction freely available. During digestion, biocytin (biotinyl-lysine) is released from the proteins and can be resorbed as easily as biotin from the intestinal tract. Afterwards, biotin is released from biocytin by the enzyme biocytinase in erythrocytes and plasma. It is then available as prosthetic group for a series of biotin-dependent enzymes.

The daily requirements of biotin are difficult to estimate because a healthy intestinal flora supplies a major part of biotin by endogenous synthesis. The generally recommended daily dose for adults is 100–200 µg. Chronic hemodialysis patients show clear improvements of neuropathological status and glucose metabolism when supplemented with biotin in a milligram range.

Biotin deficiency can be caused by e. g. destruction of the intestinal flora or extreme diets (e. g. frequent consumption of raw eggs). It can lead to dermatitis, hair loss, anorexia, mucular hypotonia, depression and reproduction problems.

3. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity
K 8141	PLATE	Microtiter plate, pre-coated	12 x 8 wells
K 0001.C.100	WASHBUF	Wash buffer concentrate, 10 x	2 x 100 ml
K 8141	CONJ	Conjugate, ready-to-use	1 x 13 ml
K 8141	STD	Standards, ready-to-use (see specification for concentration)	1 x 6 vials
K 8141	CTRL A	Control, ready-to-use (see specification for concentration)	1 x 1 vial
K 8141	CTRL B	Control, ready-to-use (see specification for concentration)	1 x 1 vial
K 8141	SAMPLEBUF	Sample dilution buffer, ready-to-use	1 x 30 ml
K 0002.15	SUB	Substrate, ready-to-use	1 x 15 ml

Cat. No.	Label	Kit components	Quantity
K 0003.15	STOP	Stop solution, ready-to-use	1 x 15 ml
K 8141	FOL	Lightproof foil to cover the microtiter plate	3 x 1 piece

For reorders of single components, use the catalogue number followed by the label as product number.

4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultrapure water*
- Calibrated precision pipettors and 10–1000 µl single-use tips
- Vortex
- Microtiter plate reader (required filters see chapter 7)

* Immundiagnostik AG recommends the use of ultrapure water (water type 1; ISO 3696), which is free of undissolved and colloidal ions and organic molecules (free of particles > 0.2 µm) with an electrical conductivity of 0.055 µS/cm at 25 °C (≥ 18.2 MΩ cm).

5. STORAGE AND PREPARATION OF REAGENTS

- Reagents with a volume less than **100 µl** should be centrifuged before use to avoid loss of volume.
- **Preparation of the wash buffer:** The **wash buffer concentrate (WASHBUF)** has to be diluted with ultrapure water **1:10** before use (100 ml WASHBUF + 900 ml ultrapure water), mix well. Crystals could occur due to high salt concentration in the concentrate. Before dilution, the crystals have to be redissolved at room temperature or in a water bath at 37 °C. The **WASHBUF** is stable at **2–8 °C** until the expiry date stated on the label. **Wash buffer** (1:10 diluted WASHBUF) can be stored in a closed flask at **2–8 °C for 1 month**.
- All other test reagents are ready-to-use. Test reagents are stable until the expiry date (see label) when stored at **2–8 °C**.

6. STORAGE AND PREPARATION OF SAMPLES

Sample storage

Serum and plasma

Plasma or serum can be stored for 5 days at room temperature (15–30 °C), 4 weeks at 2–8 °C or 21 months at -20 °C. The samples can be frozen and thawed again for up to 6 times.

Sample preparation

Serum and plasma

Human serum/plasma can directly be processed, but it should be free from insoluble particles. Remove insoluble particles by short centrifugation (3000 *g*).

Samples with an expected high biotin concentration (more than 1100 ng/l) have to be diluted appropriately with sample dilution buffer.

E. g. **dilution factor 2**: 1+1 = 1:2 = 75 µl sample + 75 µl sample dilution buffer

Urine

Urine samples have to be diluted 1:40–1:80 with sample dilution buffer before being assayed.

Milk

Milk samples have to be diluted 1:200–1:400 with sample dilution buffer before being assayed.

7. ASSAY PROCEDURE

Principle of the test

This test is a competitive ELISA for the determination of biotin in human serum.

Samples, standards and controls are pipetted into the wells (pre-coated with streptavidine) and incubated. After a washing step, conjugate (enzyme-labelled biotin) is added and competes against the biotin in the samples, standards and controls for streptavidin on the microtiter plate. Unbound enzyme-labelled biotin is washed away and the enzyme substrate TMB is added, resulting in a colour reaction. Finally, the reaction is terminated by an acidic stop solution causing a colour change from blue to yellow. The color intensity is inversely proportional to the biotin concentration. A dose response curve of the absorbance unit (optical density, OD at 450 nm)

vs. concentration is generated using the values obtained from the standards. Biotin present in the samples is determined from this curve.

Test procedure

Bring all **reagents and samples to room temperature** (20–30 °C) and mix well.

Mark the positions of standards/samples/controls on a protocol sheet.

Take as many microtiter strips as needed from the kit. Store unused strips covered with foil (FOL) and together with the desiccant bag in the closed aluminium packaging at 2–8 °C. Strips are stable until expiry date stated on the label.

For automated ELISA processors, the given protocol has to be adjusted according to the specific features of the respective automated platform. For further details please contact your supplier or Immundiagnostik AG.

Attention: Always wear gloves when working with standards and controls to avoid contamination of standards and controls by unprotected skin, especially when transferring to DSX vials.

We recommend to carry out the tests in duplicate.

1.	Add each **50 µl standards/controls/samples** into the respective wells.
2.	Cover the strips with the provided foil (FOL) and incubate for **30 min** at room temperature (20–30 °C).
3.	Discard the content of each well and wash **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
4.	Add **50 µl conjugate** (CONJ) into each well.
5.	Cover the strips with the provided foil (FOL) and incubate for **30 min** at room temperature (20–30 °C).
6.	Discard the content of each well and wash **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
7.	Add **100 µl substrate** (SUB) into each well.
8	Incubate for **10–15 minutes*** at room temperature (20–30 °C) in the **dark**.
9.	Add **100 µl stop solution** (STOP) into each well and mix well.

10.	Determine **absorption immediately** with an ELISA reader at **450 nm** against 620 nm (or 690 nm) as a reference. If no reference wavelength is available, read only at 450 nm. If the extinction of the highest standard exceeds the range of the photometer, absorption must be measured immediately at **405 nm** against 620 nm as a reference.

* The intensity of the colour change is temperature sensitive. We recommend observing the colour change and stopping the reaction upon good differentiation.

8. RESULTS

The following algorithms can be used alternatively to calculate the results. We recommend using the point-to-point calculation.

1. Point-to-point calculation

We recommend a linear ordinate for the optical density and a linear abscissa for the concentration.

2. 4 parameter algorithm

It is recommended to use a linear ordinate for the optical density and a logarithmic abscissa for the concentration. When using a logarithmic abscissa, the zero standard must be specified with a value less than 1 (e. g. 0.001).

The plausibility of the duplicate values should be examined before the automatic evaluation of the results. If this option is not available with the programme used, the duplicate values should be evaluated manually.

Serum/plasma

The obtained results do not have to be further calculated.

Urine

The obtained results have to be multiplied by the **dilution factor** used (1:40–1:80).

Milk

The obtained results have to be multiplied by the **dilution factor** used (1:200–1:400).

In case **another dilution factor** has been used, multiply the obtained result by the dilution factor used.

9. LIMITATIONS

Samples with concentrations above the measurement range (> 1100 ng/l) can be further diluted with sample dilution buffer and re-assayed. Please consider this greater dilution when calculating the results.

Samples with concentrations lower than the measurement range (= LoQ, 48,1 ng/l) cannot be clearly quantified.

10. QUALITY CONTROL

Immundiagnostik AG recommends the use of external controls for internal quality control, if possible.

Control samples should be analysed with each run. Results, generated from the analysis of control samples, should be evaluated for acceptability using appropriate statistical methods. The results for the patient samples may not be valid if within the same assay one or more values of the quality control sample are outside the acceptable limits.

Reference range

Based on Immundiagnostik AG studies of samples of apparently healthy persons (n = 40), the following ranges were estimated:

Serum/plasma

Healthy:	> 250 ng/l
Suboptimal status:	100–250 ng/l
Vitamin deficiency:	< 100 ng/l

We recommend each laboratory to establish its own reference range.

To assure a correct diagnosis of biotin deficiency, we recommend to analyse the biotin level on several consecutive days of a patient, as biotin level undergoes daily fluctuations of up to 100 %. Especially supplementation can cause the biotin level to increase tremendously in a very short time.

Urine

Adequate biotin supply is considered to start at levels of 70 nmol/l (equals 17 101,7 ng/l) and higher[14].

11. PERFORMANCE CHARACTERISTICS

All values of the following performance characteristics have been calculated using the point-to-point calculation.

Accuracy - Precision

Repeatability (Intra-Assay); n = 40

The repeatability was assessed with 2 serum samples under **constant** parameters (same operator, measurement system, day and kit lot).

Sample	Mean value [ng/l]	CV [%]
1	140.63	6.0
2	454.46	6.7

Reproducibility (Inter-Assay); n = 15

The reproducibility was assessed with 2 serum samples under **varying** parameters (different operators, measurement systems, days and kit lots).

Sample	Mean value [ng/l]	CV [%]
1	148.70	10.9
2	492.33	4.8

Analytical sensitivity

The following values have been estimated based on the concentrations of the standard without considering possibly used sample dilution factors.

Limit of blank, LoB	25 ng/l
Limit of detection, LoD	32.4 ng/l
Limit of quantitation, LoQ	48.1 ng/l
Measuring range:	48,1–1100 ng/l

The evaluation was performed according to the CLSI guideline EP-17-A2.

Analytical specificity

The specificity of the antibody was tested by measuring the cross-reactivity against a biocytin. The specificity is calculated in percent, based on the cross-reactivity of biocytin compared to biotin.

- Biotin 100.0 %
- Biocytin 67.0 %

Accuracy - Trueness

The trueness states the closeness of the agreement between the result of a measurement and the true value of the measurand. Therefore, biotin spikes with known concentrations were added to 3 different plasma samples. The results below were obtained without consideration of the sample dilution factor.

Sample [ng/l]	Spike [ng/l]	Expected [ng/l]	Obtained [ng/l]	Recovery R [%]
51.3	100	151.3	164.8	109.0
	200	251.3	289.4	115.2
	400	451.3	492.8	109.2
	600	651.3	644.4	98.9
	800	851.3	900.0	105.7
	900	951.3	964.6	101.4
	1000	1051.3	1007.7	95.9
	1100	1151.3	1032.3	89.7
	1200	1251.3	1121.5	89.6
118.8	100	218.8	214.7	98.1
	200	318.8	324.1	101.7
	400	518.8	578.7	111.5
205.7	100	305.7	317.9	104.0
	200	405.7	464.5	114.5
	400	605.7	609.0	100.5

Analytical specificity – Interferences

Different substances that might interfere when using the IDK® Biotin ELISA were tested. Therefore, positive as well as negative serum samples were supplemented with either drugs (maximum daily dose) or serum components (doses according to CLSI guideline EP7-A2) and then measured.

No interferences with the following serum components were found: Hemoglobin, bilirubin or triglycerides.

Linearity

The linearity states the ability of a method to provide results proportional to the concentration of analyte in the test sample within a given range. This was assessed according to CLSI guideline

EP06-A with a serial dilution of 3 different plasma samples.

For biotin in serum and plasma, the method has been demonstrated to be linear from 63.3 to 1134.8 ng/l based on the standard curve without considering possibly used sample dilution factors, showing a non-linear behaviour of less than ±20 % in this interval.

Sample	Dilution	Expected [ng/l]	Obtained [ng/l]	Recovery R [%]
1	undiluted	1134.8	1134.8	100.0
	1:2	567.4	652.2	114.9
	1:4	283.7	288.8	101.8
	1:8	141.85	141.5	99.8
2	undiluted	798.9	798.9	100.0
	1:2	399	390.4	97.8
	1:4	199.7	191.4	95.8
	1:8	99.86	98.9	99.0
3	undiluted	506.2	506.2	100.0
	1:2	253.1	236.7	93.5
	1:4	126.6	115.2	91.0
	1:8	63.3	55.5	87.7

12. PRECAUTIONS

- All reagents in the kit package are for *in vitro* diagnostic use only.
- Human materials used in kit components were tested and found to be negative for HIV, Hepatitis B and Hepatitis C. However, for safety reasons, all kit components should be treated as potentially infectious.
- Kit reagents contain sodium azide or ProClin as bactericides. Sodium azide and ProClin are toxic. Substrates for the enzymatic color reactions are toxic and carcinogenic. Avoid contact with skin or mucous membranes.

- The stop solution consists of diluted sulphuric acid, a strong acid. Although diluted, it still must be handled with care. It can cause burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spill should be wiped up immediately with copious quantities of water. Do not breath vapour and avoid inhalation.

13. TECHNICAL HINTS

- Do not interchange different lot numbers of any kit component within the same assay. Furthermore we recommend not assembling wells of different microtiter plates for analysis, even if they are of the same batch.
- Control samples should be analysed with each run.
- Reagents should not be used beyond the expiration date stated on kit label.
- Substrate solution should remain colourless until use.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Avoid foaming when mixing reagents.
- Do not mix plugs and caps from different reagents.
- The assay should always be performed according to the enclosed manual.

14. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- This assay was produced and distributed according to the IVD guidelines of 98/79/EC.
- The guidelines for medical laboratories should be followed.
- *IDK*® is a trademark of Immundiagnostik AG.
- 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. Immundiagnostik AG can therefore not be held responsible for any damage resulting from incorrect use.
- Warranty claims and complaints regarding deficiencies must be logged within 14 days after receipt of the product. The product should be send to Immundiagnostik AG along with a written complaint.

15. REFERENCES

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Used symbols: