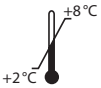
Arbeitsanleitung / Manual

IDK® Biotin ELISA

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

Gültig ab / Valid from 2016-05-19

REF **K 8141**



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EAGLE
BIOSCIENCES

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1. INTENDED USE

This Immundiagnostik 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 bacterial, 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	12 x 8 wells
K 8141	WASHBUF	ELISA wash buffer concentrate, 10 x	2 x 100 ml
K 8141	CONJ	Conjugate, ready-to-use	1 x 8 ml
K 8141	STD	Standards, ready-to-use (see specification for concentration)	6 vials
K 8141	CTRL A	Control, ready-to-use (see specification for concentration)	1 vial
K 8141	CTRL B	Control, ready-to-use (see specification for concentration)	1 vial
K 8141	SAMPLEBUF	Sample dilution buffer, ready-to-use	10 ml
K 8141	SUB	TMB substrate, ready-to-use	1 x 15 ml

Cat. No.	Label	Kit components	Quantity
K 8141	STOP	ELISA stop solution, ready-to-use	1 x 15 ml
K 8141	FOL	Lightproof foil to cover the microtiter plate	2 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

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

* Immundiagnostik AG recommends the use of Ultra Pure 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 ultra pure water **1:10** before use (100 ml WASHBUF + 900 ml ultra pure water), mix well. Crystals could occur due to high salt concentration in the stock solutions. The crystals must be redissolved at room temperature or in a water bath at 37 °C before dilution of the buffer solutions. 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 one month**.
- All other test reagents are ready to use. Test reagents are stable until the expiry date (see label of test package) when stored at **2–8 °C**.

6. STORAGE AND PREPARATION OF SAMPLES

Sample storage

Serum and plasma

Serum samples can be stored at 2–8 °C for up to 24 hours. For long time storage, serum samples should be frozen at -20 °C. Repeated freezing and thawing should be avoided.

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 kit. Store unused strips covered at 2–8 ° C. Strips are stable until expiry date stated on the label.

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

We recommend to carry out the tests in duplicate.

1.	Add each **50 µl standards/samples/controls** 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 contents of each well and wash **5 times** with **250 µl wash buffer**. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper.
4.	Add **50 µl conjugate** 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 contents of each well and wash **5 times** with **250 µl wash buffer**. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper.
7.	Add **100 µl substrate** (SUB) in each well.
8	Incubate for **10–15 minutes*** at room temperature (20–30 °C) in the dark.
9.	Add **100 µl** STOP (ELISA stop solution) 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 color change is temperature sensitive. We recommend observing the color 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.

Urin

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

Milk

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

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

9. LIMITATIONS

Samples with concentrations above the measurement range (> 1100 ng/l) must 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 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 studies of samples of apparently healthy persons (n = 40), the following ranges were estimated:

Healthy:	≥ 250 ng/l
Suboptimal status:	100–249 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 as biotin 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.

Precision and reproducibility

Intra-Assay (n = 40)

Sample	Biotin [ng/l]	CV [%]
1	140,6	6,0
2	454,5	6,7

Inter-Assay (n = 10)

Sample	Biotin [ng/l]	CV [%]
1	148,7	8,8
2	492,3	4,1

Dilution recovery

Three samples were diluted with sample dilution buffer and used in the test. The results are shown in the following table.

Sample	Dilution	Measured [ng/l]	Expected [ng/l]	Recovery R [%]
A	undiluted	1134,8	1134,8	
	1:2	652,2	567,4	114,0
	1:4	288,8	283,7	102,0
	1:8	141,5	141,9	99,7
B	undiluted	798,9	798,9	
	1:2	390,4	399,0	97,7
	1:4	191,4	199,7	95,8
	1:8	98,9	99,9	99,0
C	undiluted	506,2	506,2	
	1:2	236,7	253,1	93,5
	1:4	115,2	126,6	91,0
	1:8	55,5	63,3	87,6

Acceptance criteria for dilution recovery: R = 85–115 %.

Analytical Sensitivity

Limit of blank, LoB	25 ng/l
Limit of detection, LoD	32,4 ng/l
Limit of quantitation, LoQ	48,1 ng/l
Measurement range	48,1–1100 ng/l

The evaluation was performed according to the CLSI guideline EP-17-A2. The specified accuracy goal for the LoQ was 20 % CV.

Specificity

The specificity of the assay 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 %

Spiking Recovery

This table shows the revovery rate of biotin which was added to 2 different serum samples. The table shows the average target values and obtained values.

Sample	Spike [ng/l]	Expected [ng/l]	Measured [ng/l]	Recovery R [%]
A	0		118,8	
	100	218,8	214,7	98,5
	200	318,8	324,1	101,9
	400	518,8	578,7	111,7
B	0		205,7	
	100	305,7	317,8	103,9
	200	405,7	464,5	114,4
	400	605,7	609,0	100,5

Acceptance criteria for spike revorery rate: R = 85–115 %.

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 analyzed 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 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:


Temperature limitation

REF Catalogue Number

IVD In Vitro Diagnostic Medical Device

→REF To be used with


Manufacturer

Contains sufficient for <n> tests

LOT Lot number


Use by


Attention