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Manual

IDK® prealbumin ELISA

For the in vitro determination of prealbumin (transthyretin) in serum, plasma, urine and stool

Valid from 2020-05-20

REF **K 6331**

Σ 96

+8 °C
+2 °C

IVD

CE

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1. INTENDED USE

This assay is an ELISA intended for the quantitative determination of prealbumin (transthyretin) in serum, plasma, urine and stool. For *in vitro* diagnostic use only.

2. INTRODUCTION

Prealbumin (transthyretin) is a transport protein secreted in the liver, brain and retina. It transports thyroxin (T_4) and retinol binding protein in serum and cerebrospinal fluid. Its names are derived from its function (***trans****ports* ***thy****roxin and* ***retin****ol*) and the fact that it runs in front of albumin (*prealbumin*) in electrophoresis gels.

Prealbumin (transthyretin) is a 55 kDa homotetramer with a dimer of dimers quaternary structure. Each monomer is a 127-residue polypeptide rich in beta sheets. Association of two monomers forms a dimer, two dimers produce the homotetrameric structure and create the two thyroxine binding sites per tetramer.

Transthyretin is reduced in inflammatory reactions and is therefore a negative acute-phase protein. A mutation in the transthyretin gene can result in a chronically lowered transthyretin level in the blood, leading to amyloidosis. As transthyretin is the protein with the highest proportion of essential amino acids and has a rather short biological half-life of 1–2 days, it can be used for the diagnosis of malnourishment and disturbances of the metabolic pathways in the liver.

Indications

- Amyloidosis
- Malnourishment
- Disturbances of the metabolic pathways in the liver

3. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity
K 6331	PLATE	Microtiter plate, pre-coated	12 x 8 wells
K 0001.C.100	WASHBUF	Wash buffer concentrate, 10 x	2 x 100 ml
K 6331	CONJ	Conjugate concentrate, peroxidase-labelled	1 x 50 µl
K 6331	CONJBUF	Conjugate dilution buffer, ready-to-use	1 x 15 ml
K 6331	STD	Standards, lyophilised (see specification for concentrations)	4 x 5 vials

Cat. No.	Label	Kit components	Quantity
K 6331	CTRL1	Control, lyophilised (see specification for range)	4 x 1 vial
K 6331	CTRL2	Control, lyophilised (see specification for range)	4 x 1 vial
K 6331	SAMPLEBUF	Sample dilution buffer, ready-to-use	2 x 100 ml
K 0002.15	SUB	Substrate (tetramethylbenzidine), ready-to-use	1 x 15 ml
K 0003.15	STOP	Stop solution, ready-to-use	1 x 15 ml

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

4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultrapure water*
- Stool sample application system such as cat. no.: K 6998SAS
- Calibrated precision pipettors and 10–1000 µl single-use tips
- Foil to cover the microtiter plate
- Horizontal microtiter plate shaker
- Multi-channel pipets or repeater pipets
- Centrifuge, 3000 *g*
- Vortex
- Standard single-use laboratory glass or plastic vials, cups, etc.
- 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

- To run the assay more than once, ensure that reagents are stored at the conditions stated on the label. **Prepare only the appropriate amount necessary for each run.** The kit can be used up to 4 times within the expiry date stated on the label.
- 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**.

- The **lyophilised standards (STD)** and **controls (CTRL)** are stable at **2–8 °C** until the expiry date stated on the label. **Reconstitution** details are given in the **specification data sheet**. **Standards and controls** (reconstituted STD and CTRL) **are not stable and cannot be stored.**
- **Preparation of the conjugate:** Before use, the **conjugate concentrate (CONJ)** has to be diluted **1:500** in **conjugate dilution buffer (CONJBUF)** (20 µl CONJ + 10 ml CONJBUF). The **CONJ** is stable at **2–8 °C** until the expiry date stated on the label. **Conjugate** (1:500 diluted CONJ) **is not stable and cannot be stored.**
- 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

Serum / plasma samples

Sample storage

Fresh collected blood should be centrifuged within one hour. Store samples at -20 °C if not assayed on the same day. Lipemic or hemolytic samples may give erroneous results. Samples should be mixed well before assaying.

Sample dilution

Standard serum and plasma samples are diluted 1:20 000 before being assayed. The dilution can be performed in three steps, e. g.:

- **50 µl** sample + **950 µl** sample dilution buffer (SAMPLEBUF), mix well
  = **1:20** (**dilution I**)
- **100 µl** dilution I + **900 µl** sample dilution buffer, mix well
  = **1:10** (**dilution II**)
- **10 µl** dilution II + **990 µl** sample dilution buffer, mix well
  = **1:100** (**dilution III**).
  This results in a **final dilution of 1:20 000**.

For analysis, pipet **100 µl** of **dilution III** per well.

Note: We recommend the determination of the optimal sample dilution (1:10 000–1:30 000) in a preliminary experiment.

Stool samples

Extraction of the stool samples

Wash buffer (1:10 diluted WASHBUF) is used as a **sample extraction buffer**. We recommend the following sample preparation:

Stool Sample Application System (SAS) (Cat. No.: K 6998SAS)

Stool sample tube – Instructions for use

Please note that the dilution factor of the final stool suspension depends on the amount of stool sample used and the volume of the buffer.

SAS with 1.5 ml sample extraction buffer:

Applied amount of stool:	15 mg
Buffer Volume:	1.5 ml
Dilution Factor:	1:100

Please follow the instructions for the preparation of stool samples using the SAS as follows:

a) The raw stool sample has to be thawed. For particularly heterogeneous samples we recommend a mechanical homogenisation using an applicator, inoculation loop or similar device.

b) Fill the **empty stool sample tube** with 1.5 ml **sample extraction buffer** (1:10 diluted WASHBUF) before using it with the sample. **Important:** Allow the sample extraction buffer to reach room temperature.

c) Unscrew the tube (yellow part of cap) to open. Insert the yellow dipstick into the sample. The lower part of the dipstick has notches which need to be covered completely with stool after inserting it into the sample. Place dipstick back into the tube. When putting the stick back into the tube, excess material will be stripped off, leaving 15 mg of sample to be diluted. Screw tightly to close the tube.

d) Vortex the tube well until no stool sample remains in the notches. **Important:** Please make sure that you have a maximally homogenous suspension after shaking. Especially with more solid samples, soaking the sample in the tube with sample extraction buffer for ~ 10 minutes improves the result.

e) Allow the sample to stand for ~10 minutes until sediment has settled. Floating material like shells of grains can be neglected.

f) Carefully unscrew the complete cap of the tube including the blue ring plus the dipstick. Discard cap and dipstick. Make sure that the sediment will not be dispersed again.

Dilution I: **1:100**

Sample dilution

The suspension of the sample preparation procedure (dilution I) is diluted **1:20 in wash buffer**. For example:

- **50 µl** supernatant (dilution I) + **950 µl** wash buffer, mix well
  = **1:20** (**dilution II**)
  This results in a **final dilution of 1:2 000**.

For analysis, pipet **100 µl** of **dilution III** per well.

7. ASSAY PROCEDURE

Principle of the test

This ELISA is designed for the quantitative determination of human prealbumin (transthyretin) in serum, plasma, urine and stool.

Standards, controls and samples containing human prealbumin (transthyretin) are added to the wells of a microplate coated with polyclonal anti-human prealbumin antibodies. The antibodies immobilised on the walls of the microtiter wells capture prealbumin in the samples during the first incubation step. After washing away the unbound components, a peroxidase-conjugated anti-prealbumin detection antibody is added to each well. During a second incubation, the detection antibody is bound to the captured prealbumin. A sandwich of capture antibody – human prealbumin – peroxidase conjugate is formed. Tetramethylbenzidine (TMB) is used as a peroxidase substrate. Finally, an acidic stop solution is added to terminate the reaction. The intensity of the yellow colour is directly proportional to the prealbumin concentration of the sample. A dose response curve of the absorbance unit (optical density, OD at 450 nm) vs. concentration is generated, using the values obtained from the standard. Prealbumin (transthyretin), present in the samples, is determined directly from this curve.

Test procedure

Bring all **reagents and samples to room temperature** (15–30 °C) and mix well.

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

Take as many microtiter strips as needed from the kit. Store unused strips together with the desiccant bag in the closed aluminium packaging at 2–8 °C. Strips are stable until the 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.	**Before use**, wash the wells **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
2.	Add each **100 µl standards/controls/diluted samples** into the respective wells.
3.	Cover the strips and incubate for **1 h** at room temperature (15–30 °C) on a **horizontal shaker***.
4.	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.
5.	Add **100 µl conjugate** (diluted CONJ) into each well.
6.	Cover the strips and incubate for **1 h** at room temperature (15–30 °C) on a **horizontal shaker***.
7.	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.
8.	Add **100 µl substrate** (SUB) into each well.
9.	Incubate for **10–20 min**** at room temperature (15–30 °C) in the **dark**.
10.	Add **100 µl stop solution** (STOP) into each well and mix well.

11.	Determine **absorption immediately** with an ELISA reader at **450 nm** against 620 nm (or 690 nm) as a reference. 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.

* We recommend shaking the strips at 550 rpm with an orbit of 2 mm.

** 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 4 parameter algorithm.

1. 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).

2. Point-to-point calculation

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

3. Spline algorithm

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

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 samples

The obtained results have to be multiplied by the **dilution factor of 20 000** to get the actual concentrations.

Stool samples

The obtained results have to be multiplied by the **dilution factor of 2 000** to get the actual concentrations.

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 (see definition below) can be further diluted and re-assayed. Please consider this higher dilution when calculating the results.

Samples with concentrations lower than the measurement range (see definition below) cannot be clearly quantified.

The upper limit of the measurement range can be calculated as:

highest concentration of the standard curve × *sample dilution factor to be used*

The lower limit of the measurement range can be calculated as:

Analytical sensitivity × *sample dilution factor to be used*

Analytical sensitivity see chapter "Performance Characteristics".

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 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

We recommend each laboratory to establish its own reference range.

11. PERFORMANCE CHARACTERISTICS

Precision and reproducibility

Two sera were diluted 1:10 000 or 1:20 000 and measured using the assay.

Intra-Assay (n = 24)

Sample	Prealbumin [µg/ml]	CV [%]
1	196.6	3.8
2	117.8	3.0

Inter-Assay (n = 14)

Sample	Prealbumin [µg/ml]	CV [%]
1	193.0	6.5
2	117.3	4.6

Analytical Sensitivity

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

The zero standard was measured 20 times. The detection limit was set as B_0 + 2 SD and estimated to be 0.933 ng/ml.

Specificity

As determined by rocket immunoelectrophoresis, the antibody cross-reacts with the protein equivalent to prealbumin in cat, dog, goat, horse, sheep and swine and shows no cross-reaction with its equivalent in guinea pig, mouse and rat.

- < 0,1% with sIgA
- < 0,1% with β-2-M
- < 0,1% with human Albumin

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 colour 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 should 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 the 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

General literature

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3. Kovesdy CP, Kalantar-Zadeh K. Accuracy and limitations of the diagnosis of malnutrition in dialysis patients. Seminars in dialysis. 2012 Jul;25(4):423–7.

Literature using the IDK® prealbumin (transthyretin) ELISA

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5. Yılmaz D, Sönmez F, Karakaş S, Yavaşcan Ö, Aksu N, Ömürlü İK, et al. Evaluation of Nutritional Status in Children during Predialysis, or Treated By Peritoneal Dialysis or Hemodialysis. Journal of Tropical Pediatrics. 2016;fmv094.
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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

 Consult instructions for use

 Consult specification data sheet