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Manual

Albumin ELISA

Super sensitive

For the in vitro determination of albumin in urine and stool

Valid from 2026-06-26

REF **K 6330**

Σ 96

+8 °C
+2 °C

IVD

CE

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1. INTENDED USE

This Immundiagnostik AG assay is an enzyme immunoassay intended for the quantitative determination of albumin in urine and stool. For *in vitro* diagnostic use only.

2. INTRODUCTION

Albumin is the major protein in human plasma (40–60 %). It is synthesised in the liver depending on the protein uptake. Substantially, changes in the concentration of albumin in urine and faeces are a result of distribution disorder, less synthesis problems. In the presence of protein malnutrition, the extent of edema only weakly correlates with the albumin concentration. Stronger albumin loss leads to increased synthesis, for example in case of nephrotic syndrome.

Elevated levels of albumin and hemoglobin in stool are observed not only in colorectal carcinomas, but also in polyps and during chronic inflammatory diseases (Morbus Crohn or Colitis Ulcerosa). Albumin in faecal samples refers to an inflammatory reaction combined with intestinal bleeding.

Indications

- Detection of source of bleeding in the lower gastrointestinal tract
- Detection of colorectal carcinoma
- Investigation of high risk patients
- Morbus Crohn, Colitis Ulcerosa

3. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity
K 6330	PLATE	Microtiter plate, pre-coated	12 x 8 wells
K 0001.C.100	WASHBUF	Wash buffer concentrate, (10x)	1 x 100 ml
K 6330	SAMPLEBUF	Sample dilution buffer, ready-to-use	1 x 100 ml
K 6330	CONJBUF	Conjugate dilution buffer, ready-to-use	1 x 15 ml
K 6999.C.100	IDK Extract®	Extraction buffer concentrate *IDK Extract®*, 2.5x	1 x 100 ml
K 6330	CONJ	Conjugate concentrate, peroxidase-labelled (goat-anti-albumin)	1 x 200 µl
K 6330	STD	Standards, lyophilised (0, 12.5, 50, 200, 800 µg/l)	4 x 5 vials

Cat. No.	Label	Kit components	Quantity
K 6330	CTRL1	Control 1, lyophilised (see specification for range)	4 x 1 vial
K 6330	CTRL2	Control 2, lyophilised (see specification for range)	4 x 1 vial
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 x *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 (e. g. 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**.

- **Preparation of the extraction buffer:** The **extraction buffer concentrate *IDK Extract*®** has to be diluted with ultrapure water **1:2.5** before use (e. g. 100 ml *IDK Extract*® + 150 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 37 °C in a water bath. The ***IDK Extract*®** is stable at **2–8 °C** until the expiry date stated on the label. **Extraction buffer** (1:2.5 diluted *IDK Extract*®) can be stored in a closed flask at **2–8 °C for 4 months**.
- The **lyophilised standards (STD)** and **controls (CTRL)** are stable at **2–8 °C** until the expiry date stated on the label. Before use, the STD and CTRL have to be reconstituted with **500 µl of ultrapure water** and mixed by gentle inversion to ensure complete reconstitution. Allow the vial content to dissolve for 10 minutes and then mix thoroughly. **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 **1:101** in **conjugate dilution buffer** (e. g. 100 µl CONJ + 10 ml CONJBUF). The CONJ is stable at **2–8 °C** until the expiry date stated on the label. **Conjugate** (1:101 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

Storage of urine samples

Adjust urine to a pH 6 to 8 with 1 N NaOH. Samples can be stored for two weeks at 2–8 °C or for longer storage at -20 °C.

Dilution of urine samples

Urine must be diluted **1:200** with sample dilution buffer (SAMPLEBUF**)** before performing the assay, e.g.

10 µl sample + **1990 µl** SAMPLEBUF, mix well.

For testing in duplicates, pipette **2 x 100 µl** of each prepared sample per well.

Storage of stool samples

Raw stool

Raw stool is stable for 2 days at 2-8 °C. It can be stored for up to 1 month at -20 °C. Avoid more than 3 freeze-thaw-cycles

Stool extract

Stool extract is stable for 1 day at room temperature. It can be stored up to 9 days at 2–8 °C or -20 °C. They can be frozen and thawed again for up to 3 times.

Extraction of the stool samples

Extraction buffer (1:2.5 diluted *IDK Extract®*) 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** (1:2.5 diluted *IDK Extract®*) 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 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

Dilution of stool samples

The supernatant of the sample preparation procedure (dilution I) is diluted **1:2.5 in sample dilution buffer** (SAMPLEBUF). For example:

- **200 µl** supernatant (dilution I) + **300 µl** SAMPLEBUF = **1: 2.5** (**dilution II**) . This results in a **final dilution of 1:250.**

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

7. ASSAY PROCEDURE

Principle of the test

This ELISA is designed for the quantitative determination of albumin.

This Enzyme-Linked Immunosorbent Assay (ELISA) is a two step assay for the ultra sensitive determination of human albumin in stool and urine. A polyclonal rabbit antibody specific for human albumin is immobilised on a microtiter plate and a second anti-albumin antibody is conjugated to peroxidase.

In a first incubation step, the albumin in the samples is bound to the immobilised anti-albumin antibodies. A washing step is carried out to remove all unbound substances. In a second incubation step, a peroxidase-labelled anti-albumin antibody is added. After another washing step, to remove all unbound substances, a peroxidase-substrate, tetramethylbenzidine, is added. The enzymatic reaction is terminated by an acidic stop solution, whereby the colour converts from blue to yellow. The intensity of the yellow colour is directly proportional to the concentration of albumin in 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. Albumin, present in the patient 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/samples/controls on a protocol sheet.

Take as many microtiter strips as needed from kit. Store unused strips 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 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/prepared samples** into the respective wells.
3.	Cover the strips and incubate for **1 hour** 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 hour** 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 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.

* 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.

Urine

The obtained results have to be multiplied by the **dilution factor of 200** to get the actual concentrations.

Stool

The obtained results have to be multiplied by the **dilution factor of 250** to get the actual concentrations.

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

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 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:

LoB × sample dilution factor to be used

LoB 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 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 stool samples of apparently healthy persons (n = 76), the following reference range was estimated.

Albumin in stool:	< 9.2 mg/l
Albumin in urine:	< 20 mg/l*

* Test information, Labor Limbach, Heidelberg, http://www.labor-limbach.de

We recommend each laboratory to establish its own reference range.

11. PERFORMANCE CHARACTERISTICS

Accuracy – Precision

Repeatability (Intra-Assay); n = 19

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

Sample	Mean value [mg/l]	CV [%]
1	7.29	2.2
2	26.43	2.7

Reproducibility (Inter-Assay); n = 59

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

Sample	Mean value [ng/ml]	CV [%]
1	27.30	8.0
2	144.65	6.3

Accuracy – Trueness

The trueness states the closeness of the agreement between the result of a measurement and the true value of the measurand. Therefore, albumin spikes with known concentrations were added to 6 different stool samples. The results are shown in the following table without considering possibly used sample dilution factors:

Sample [ng/ml]	Spike [ng/ml]	Expected [ng/ml]	Obtained [ng/ml]	Recovery [%]
1.78	25	26.78	33.20	123.97
1.78	25	26.78	27.70	103.43
0.82	25	25.82	29.60	114.64
11.70	25	36.70	40.60	110.63
8.71	25	33.71	40.00	118.65
15.90	25	40.90	43.10	105.38

Analytical sensitivity

The following value has been estimated based on the concentrations of the standards without considering possibly used sample dilution factors.

Limit of blank, LoB 0.976 ng/ml

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 stool samples.

For albumin in stool and urine, the method has been demonstrated to be linear from 39.97 to 385.78 ng/ml 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/ml]	Obtained [ng/ml]	Recovery [%]
A	1:1 000	385.78	385.78	100.00
	1:2 000	192.89	178.22	92.39
	1:4 000	96.45	71.17	73.79
	1:8 000	48.22	31.99	66.34
B	1:1 000	319.76	319.76	100.00
	1:2 000	159.88	156.70	98.01
	1:4 000	79.94	67.17	84.03
	1:8 000	39.97	30.73	76.88
C	1:1 000	327.54	327.54	100.00
	1:2 000	163.77	152.55	93.15
	1:4 000	81.89	64.56	78.84
	1:8 000	40.94	28.87	70.52

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 or ProClin are hazardous to health and the environment. Substrates for enzymatic colour reactions may also cause skin and/or respiratory irritation. Any contact with the substances must be avoided. Further safety information can be found in the safety data sheet, which is available from Immundiagnostik AG on request.
- The 10x Wash buffer concentrate (WASHBUF) contains surfactants which may cause severe eye irritation in case of eye contact.

  Warning: Causes serious eye irritation. **IF IN EYES:** Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. If eye irritation persists: get medical Advice/attention.
- 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*® and *IDK Extract*® are trademarks 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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2. Baltatu, Ovidiu, Cécile Cayla, Radu Iliescu, Dmitrii Andreev, and Michael Bader. 2003. "Abolition of End-Organ Damage by Antiandrogen Treatment in Female Hypertensive Transgenic Rats." Hypertension 41 (3 Pt 2): 830–33.
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4. John, M., H. Schmidt-Gayk, B. Arndt, and D. Theuer. 1994. "Nachweis von Albumin im Stuhl zur Erkennung okkulter Blutungen: Vergleich zweier immunologischer Tests. Radiale Immundiffusion vs. BM-Test Colon Albumin Screening for Occult Blood by Fecal Albumin: Comparison of Two Immunological Tests --." Klinisches Labor 40: 77–81.

Used symbols:

LOT	Lot number	REF	Catalogue number
IVD	*In Vitro* Diagnostic Medical Device	→REF	To be used with
	Manufacturer		Contains sufficient for <n> tests
	Temperature limitation		Use by
	Consult product specification data sheet		Consult instructions for use
	European Conformity		Do not re-use
	Contains plasma derivates or human blood		Irritant
UDI	Unique Device Identification		