Human Albumin ELISA KIT

Cat. No.: DEIA9947
Pkg.Size: 96T

Intended use

The Human Albumin ELISA KIT is intended for the quantitative determination of Albumin in urine and stool.

General Description

Albumin is the major protein in human plasma (40-60%). It is synthesized in the liver depending of the protein uptake. Albumin in fecal samples refers to an inflammatory reaction combined with intestinal bleeding. Elevated levels of albumin and hemoglobin in stool are found not only by colorectal carcinomas, but also with polyps and during chronic inflammatory diseases (Morbus Crohn or Colitis Ulcerosa).

Indication

1) Detection of source of bleeding in the lower gastrointestinal tract.
2) Detection of colorectal carcinoma.
3) Investigation of high risk patients.
4) Morbus Crohn, Colitis Ulcerosa.

Principle Of The Test

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 immobilized on microtiter plates and a second anti-Albumin antibody is conjugated to peroxidase (POD). Albumin in samples is bound to the immobilized antibody and after a washing step, to remove all unbound material. The quantification of the bound Albumin is carried out by adding a POD-labeled anti-Albumin antibody. After a second washing step a POD-substrate is added. The enzyme reaction is stopped and the absorbance is measured photometrically at 450 nm.

The intensity of the formed color is directly proportional to the concentration of Albumin in the samples.

Reagents And Materials Provided

1. Precoated strips, 96
2. ELISA wash buffer concentrate (10x), 1 x 100 ml
3. Sample dilution buffer, ready to use, 1 x 100 ml
4. Conjugate dilution buffer, ready-to-use, 1 x 15 ml
5. Conjugate, (rabbit-anti-Albumin peroxidase-labeled), 1 x 50 µl
6. Calibrators, lyophilized (0, 12.5, 50, 200, 800 µg/l), 4 x 5 vials
7. Control, lyophilized, 4 x 1 vial
8. TMB substrate (Tetramethylbenzidine), ready-to-use, 1 x 15 ml
9. ELISA stop solution, ready to use, 1 x 7 ml

Materials Required But Not Supplied

1. Bidistilled (aqua bidest.) and sterile water
2. Laboratory balance
3. Precision pipettors calibrated and tips to deliver 5-1000 µl
4. Foil to cover the microtiter plate
5. Horizontal microtiter plate shaker with an incubator for 37° C
6. A multi-channel dispenser or repeating dispenser
7. Centrifuge capable of 3000 x g
8. Vortex-Mixer
9. Standard laboratory glass or plastic vials, cups, etc.
10. Microtiter plate reader at 450 or 405 nm (reference wave length 620 or 690 nm)

**Storage**

1. The crystals must be redissolved at 37°C using a water bath before dilution of the buffer solutions. The WASHBUF (wash buffer concentrate) is stable at 2-8°C until the expiry date stated on the label. Diluted buffer solution can be stored in a closed flask at 2-8°C for one month.
2. The CONJ (conjugate, POD-Antibody) is stable at 2-8 °C until the expiry date given on the label. Diluted antibody is not stable and can not be stored.
3. All other test reagents are ready for use. The test reagents are stable up to the date of expiry (see label of test package) when stored at 2-8°C.

**Specimen Collection And Handling**

**Urine**
Adjust urine to a pH 6 to 8 with 1 N NaOH. Samples can be stored for two weeks at 2-8 °C or at -20°C for longtime storage. For the assay, dilute samples 1:200 with SAMPLEBUF, e.g. 10 µl sample +1990 µl SAMPLEBUF.

**Faeces**
Add about 100 mg of the sample (note the sample weight for the calculation) to 5 ml of wash buffer and mix. Centrifuge the sample suspension for 10 min at 3000 rpm. Transfer 1 ml of the supernatant into an Eppendorf tube and centrifuge once more at 13.000 rpm for 5 min. Dilute supernatant 1:5 in SAMPLEBUF (sample dilution buffer; e.g. 200 µl supernatant + 800 µl SAMPLEBUF). Use 100 µl of the obtained solution for the experiment. We recommend to weight fresh stool samples for each run. Supernatant is not stable and can not be stored. Stool samples can be stored at -20°C for 4 weeks. Avoid repeated freezing and thawing.

CD recommends the use of tubes from Roche Diagnostics/ Mannheim for sample preparation.
CD recommends commercial control samples for internal quality control. Control samples should be analyzed 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.

**Reagent Preparation**

1. To run assay more than once, ensure that reagents are stored at conditions stated on the label. Prepare only the appropriate amount necessary for each assay. The kit can be used up to 4 times within the expiry date stated on the label.
2. Reagents with a volume less than 100 µl should be centrifuged before use to avoid loss of volume.
3. The WASHBUF (wash buffer concentrate) should be diluted with aqua bidest. 1:10 before use (100 ml WASHBUF + 900 ml aqua bidest.), mix well. Crystals could occur due to high salt concentration in the stock solutions.
4. The lyophilized STD (calibrators) and the CTRL (control) must be reconstituted with 500 µl aqua bidest. Allow the vial content to dissolve for 10 minutes and mix thoroughly by gentle inversion to insure complete reconstitution. Reconstituted Calibrators and Controls are not stable and can not be stored.
5. The CONJ (conjugate, POD-Antibody) must be diluted 1:400 in CONJBUF (conjugate dilution buffer, e.g. 25 µl CONJ + 10 ml CONJBUF).
Assay Steps

Procedural notes
1) Do not interchange different lot numbers of any kit component within the same assay.
2) Quality control guidelines should be observed.
3) 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. CD AG can therefore not be held responsible for any damage resulting from wrong use.
4) The assay should always be performed according the enclosed manual.

Test procedure
Wash the precoated PLATE (microtiter plate) 5 x with 250 µl diluted wash buffer. After the final washing step, the inverted PLATE should be firmly tapped on absorbent paper to remove excess solution. This is valid for all following washing steps. Carry out each determination in duplicate for standards, controls and samples.
1. Add 100 µl STD (Standard), CTRL (Control) and pre-diluted patient samples into respective wells.
2. Incubate for 1 hour shaking on a horizontal mixer at room temperature.
3. Decant the content of the PLATE and wash the wells 5 x with 250µl diluted wash buffer.
4. Add 100 µl diluted conjugate in each well.
5. Incubate for 1 hour shaking on a horizontal mixer at room temperature.
6. Decant the content of the PLATE and wash the wells 5 x with 250µl diluted wash buffer.
7. Add 100 µl SUB (TMB substrate) in each well.
8. Incubate for 10-20 minutes at room temperature.
9. Add 50 µl STOP (stop solution) in each well and mix shortly.
10. Determine absorption immediately with an ELISA reader at 450 nm against 620 nm (or 690 nm) as reference. If no reference wavelength is available, read only at 450 nm. If the extinction of the highest standard exceeds the measurement range of the photometer, absorption must be measured immediately at 405 nm against 620 nm (or 690 nm) as reference.

Typical Standard Curve
A calibration curve is constructed from the standards. Commercially available software can be used as well as graph paper. Results of the samples are read from this calibration curve. THE CALIBRATION CURVE IS NOT LINEAR, therefore a spline-algorhythm is recommended.

Figure 1.
Faeces
For the Albumin concentration of faeces samples, calculate as described in the following example:
Weight: 80 mg (1ml stool = 1g) = 0.08 ml
Dilution step 1: 5ml / 0.08ml = 62.5
Dilution step 2: 5
Dilution factor: 312.5
Multiply the result by 312.5 to get the concentration of the sample. The dilution factor depends on the weight of the faeces.

Urine
To get the Albumin concentration in urine samples the observed values must be multiplied by 200.

Reference Values
Albumin in stool: < 9.2 mg/l (n = 76)
Albumin in urine: 5 - 16 mg/l

Sensitivity
The detection limit was set as B0 + 2 SD and estimated to be 12.5 ng/l. The Zero-standard was measured 20 times.

Specificity
No cross reactivity to other plasma proteins in faeces.

Reproducibility
Intra-Assay: 5%
Inter-Assay: 8%

Precautions
1. Quality control guidelines should be followed.
2. 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.
3. Kit reagents contain sodium azide or thimerosal as bactericides. Sodium azide and thimerosal are toxic. Substrates for the enzymatic color reactions are toxic and carcinogenic. Avoid contact with skin or mucous membranes.
4. Stop solution is composed of sulfuric acid, which is a strong acid. Even diluted, it still must be handled with care. It can cause acid burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spills should be wiped out immediately with copious quantities of water.
5. Do not mix different lot numbers of any kit component.
6. Reagents should not be used beyond the expiration date shown on the kit label.
7. The assay should always be performed according the enclosed manual.
8. 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. CD AG can therefore not be held responsible for any damage resulting from wrong use.
9. Warranty claims and complaints in respect of deficiencies must be logged within 14 days after receipt of the product. The product should be send to CD AG along with a written complaint.

Limitations
Samples with Albumin levels greater than the highest calibrator should be further diluted and re-assayed.
# Analyte Gene Information

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<th>Gene Name</th>
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<td>Official Symbol</td>
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<td>Synonyms</td>
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<td>Function</td>
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## REFERENCES