Albumin ELISA

For the quantitative determination of albumin in human urine and stool.

For Research Use Only. Not For Use In Diagnostic Procedures.

Catalog Number: 30-ALBHU-E01
Size: 96 Wells
Version: 2020-01-23- ALPCO 2.1

INTENDED USE

The Albumin ELISA is intended for the quantitative determination of albumin in human urine and stool samples. For Research Use Only. Not for use in diagnostic procedures.

PRINCIPLE OF THE ASSAY

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 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 immobilized anti-albumin antibodies. A washing step is carried out to remove all unbound substances. In a second incubation step, a peroxidase-labeled 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 color converts from blue to yellow. The intensity of the yellow color is directly proportional to the concentration of albumin in the sample. A dose response curve of the absorbance unit (optical density, OD) versus concentration is generated using the results obtained from the calibrators. Albumin in the samples is determined directly from this curve.

MATERIALS SUPPLIED

Component	Quantity	Preparation
Albumin Microplate (96 wells)	12 x 8 strips	Ready to use
Standards (1-5) (0, 12.5, 50, 200, 800 ng/mL)	4 x 5 vials each	Lyophilized
Wash Buffer Concentrate	1 x 100 mL	10X
Control Levels 1 and 2	4 x 1 vial each	Lyophilized
Sample Dilution Buffer	1 x 100 mL	Ready to use
Extraction Buffer Concentrate	1 x 100 mL	2.5X
Conjugate Dilution Buffer	1 x 15 mL	Ready to use
Conjugate Concentrate	1 x 50 µL	401X
TMB Substrate	1 x 15 mL	Ready to use
Stop Solution	1 x 15 mL	Ready to use

MATERIALS REQUIRED

- Precision pipettes for dispensing up to 1000 µL (with disposable tips)
- Repeating or multi-channel pipette for dispensing up to 1000 µL
- Volumetric containers and pipettes for reagent preparation
- Distilled/Deionized water for reagent preparation
- Microplate washer or wash bottle
- Microplate shaker capable of 550 RPM, orbit 2mm
- Microplate reader
- Centrifuge (1,000 xg to 3,000 xg)
- Vortex for sample preparation
- Laboratory Balance
- Foil to cover the microplate
- Timer

PRECAUTIONS

1. The human blood products incorporated into this kit have been tested for the presence of HIV (Human Immunodeficiency Virus), HBV (Hepatitis B Virus), and HCV (Hepatitis C Virus). Test methods for these viruses do not guarantee the absence of a virus; therefore, all reagents should be treated as potentially infectious. Handling and disposal should be in accordance with all appropriate national and local regulations for the handling of potentially biohazardous materials.
2. All materials derived from animal sources are bovine spongiform encephalopathy (BSE) negative. However, all materials should be treated as potentially infectious.
3. 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.
4. The stop solution consists of diluted sulfuric 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 out immediately with copious quantities of water. Do not breathe vapor and avoid inhalation.
5. Avoid direct contact with skin.
6. This product is not for internal use.
7. Avoid eating, drinking, or smoking when using this product.
8. Do not pipette any reagents by mouth.
9. Reagents from this kit are lot-specific and must not be substituted.
10. Do not use reagents beyond the expiration date.
11. Variations to the test procedure are not recommended and may influence the test results.

STORAGE CONDITIONS

The kit should be stored at 2-8°C. The kit is stable until the expiration date on the box label and can be used up to 4 times within the expiry date stated on the label.

SAMPLE HANDLING

Stool and urine samples are appropriate for use in this assay.

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.

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.

Extraction of the Stool Sample:

Diluted extraction buffer is used as a sample extraction buffer. It is recommended to perform the following sample preparation:

1. The raw stool sample must be thawed. For particularly heterogeneous samples, it is recommended to perform a mechanical homogenization using an applicator, inoculation loop or similar device.
2. Fill the empty sample tube with 1.5 mL of prepared extraction buffer (diluted 1:2.5) before using it with the sample. Important: Allow the extraction buffer to reach room temperature. Add 15mg of sample. If using the EZ Extraction Device, the device is provided in a prefilled format and does not require any additional extraction buffer.

3. If using a pre-filled extraction device, unscrew the tube (yellow part of cap) to open. Insert yellow dipstick into 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 and leave 15 mg of sample to be diluted. Screw tightly to close the tube.
4. Shake the tube well until no stool sample remains in the notches. Important: Please make sure that the container has a maximally homogenous suspension after shaking. Especially with more solid samples, soaking the sample in the tube with buffer for approximately 10 minutes improves the result.
5. Allow sample to stand for approximately 10 minutes until sediment has settled. Floating material like shells of grains can be neglected.
6. 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. This will result in a dilution of 1:100 (Dilution I).

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 sample dilution buffer = 1:2.5 (Dilution II).
This results in a final dilution of 1:250.

For analysis, pipet 100 µL of Dilution II per well.

Urine Samples

Storage of Urine Samples:

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

Dilution of Urine Samples

For the assay, urine samples must be diluted 1:200 with sample dilution buffer (SAMPLEBUF)

10 µL sample +1990 µL SAMPLEBUF, mix well.

For testing in duplicates, pipette 100µL of each prepared urine sample per designated well.

REAGENT PREPARATION

All reagents must be equilibrated to room temperature prior to preparation and subsequent use in the assay. Prepare enough reagents for only the number of strips used. Reagents with a volume less than 100 µL should be centrifuged before use to avoid loss of volume.

Standards and ***Controls (Levels 1 and 2)*** are provided in a lyophilized form. Before use, reconstitute in 500 µL of distilled water. Close the vial with the rubber stopper and cap, and gently invert to ensure complete reconstitution. Allow the vial contents to dissolve for 10 minutes and then mix thoroughly. The lyophilized controls are stable at 2-8°C until the expiry date stated on the label. Reconstituted standards and controls are **not** stable and cannot be stored.

Wash Buffer Concentrate (10X) must be diluted with distilled water 1:10 before use (100 mL WASHBUF + 900 mL distilled water) and mixed well. Crystals could occur due to high salt concentration in the concentrate. Before dilution, the crystals must be dissolved 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. 1X working wash buffer (1:10 diluted WASHBUF) can be stored in a closed flask at 2–8 °C for 1 month.

Extraction Buffer Concentrate (2.5X) must be diluted with distilled water 1:2.5 before use (100 mL Extraction Buffer + 150 mL distilled water), mix well. Crystals could occur due to high salt concentration in the stock solutions. Before dilution, the crystals must be dissolved at 37°C in a water bath. The Extraction Buffer is stable at 2–8 °C until the expiry date stated on the label. Prepared extraction buffer (1:2.5 diluted) can be stored in a closed flask at 2–8 °C for 4 months.

Conjugate Concentrate (401X) must be diluted 1:401 in conjugate dilution buffer (25 µL CONJ + 10 mL conjugate dilution buffer). The concentrate is stable at 2–8°C until the expiry date stated on the label. 1X working conjugate 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 of test package) when stored at 2–8 °C.

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 samples may not be valid if within the same assay one or more values of the quality control sample are outside the acceptable limits.

ASSAY PROCEDURE

All reagents and microplate strips (while sealed in foil pouch) should be equilibrated to room temperature prior to use. Gently mix all reagents before use. A standard curve must be performed with each assay run and with each microplate if more than one is used at a time. All standards, controls, and samples should be run in duplicate.

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 ALPCO.

1. Bring all reagents and samples to room temperature (15–30°C) and mix well.
2. Before use, wash the pre-coated microtiter plate 5 times with 250 µL 1X working wash buffer. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
3. Add 100 µL of standards, controls, and samples into the respective wells. See *Reagent Preparation* for control reconstitution instructions. A suggested plate layout is included.
4. Cover plate tightly and incubate for 1 hour at room temperature (15–30°C) on a horizontal shaker set to 550 RPM, orbit 2mm.
5. Discard the contents of each well. Wash each well 5 times with 250µL of 1X working wash buffer. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
6. Add 100 µL of prepared conjugate into each well.
7. Cover plate tightly and incubate for 1 hour at room temperature (15–30°C) on a horizontal shaker set to 550 RPM, orbit 2mm.
8. Discard the contents of each well. Wash each well 5 times with 250 µL of 1X working wash buffer. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
9. Add 100µL of substrate into each well.
10. Incubate for 10–20 minutes at room temperature (15–30°C) in the dark.*
11. Add 100 µL of stop solution into each well, mix thoroughly.
12. Determine absorption immediately with an ELISA reader at 450nm against 620nm (or 690nm) as a reference. If no reference wavelength is available, read only at 450nm. If the extinction of the highest standard exceeds the range of the photometer, absorption must be measured immediately at 405nm against 620nm as a reference.

* The intensity of the color change is temperature sensitive. It is recommended to observe the color change and to stop the reaction upon good differentiation.

CALCULATION OF RESULTS

The following algorithms can be used alternatively to calculate the results. It is recommended to use 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 calibrator must be specified with a value less than 1 (e.g. 0.001).
2. Point-to-point calculation: It is recommended to use a linear ordinate for the optical density and a linear abscissa for the concentration.
3. Spline algorithm: It is recommended to use a linear ordinate for the optical density and a linear abscissa for the concentration.

The plausibility of the pairs of values should be examined before the automatic evaluation of the results. If this option is not available with the program used, a comparison of the paired values should be done manually.

Stool
Multiply the obtained results by the dilution factor of 250 to get the actual concentrations.

Urine
Multiply the obtained results by the dilution factor of 200 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.

LIMITATIONS

Samples with concentrations above the measurement range (see definition below) can be further diluted and re-assayed. 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 x sample dilution factor to be used

The lower limit of the measurement range can be calculated as:
Analytical sensitivity x sample dilution factor to be used

PERFORMANCE CHARACTERISTICS

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   0.98 ng/mL

Accuracy- Precision

Repeatability intra-assay; n=19
The repeatability was assessed with 2 stool samples under constant parameters (same operator, measurement system, day, and kit lot).

	Sample 1	Sample 2
Mean	7.3 mg/L	26.4 mg/L
CV (%)	2.2	2.7
n	19	19

Reproducibility (inter-assay); n =59
The reproducibility was assessed with 2 control samples under varying parameters (different operators, measurement systems, days, and kit lots).

	Sample 1	Sample 2
Mean	27.3 ng/mL	144.7 ng/mL
CV (%)	8.0	6.3
n	59	59

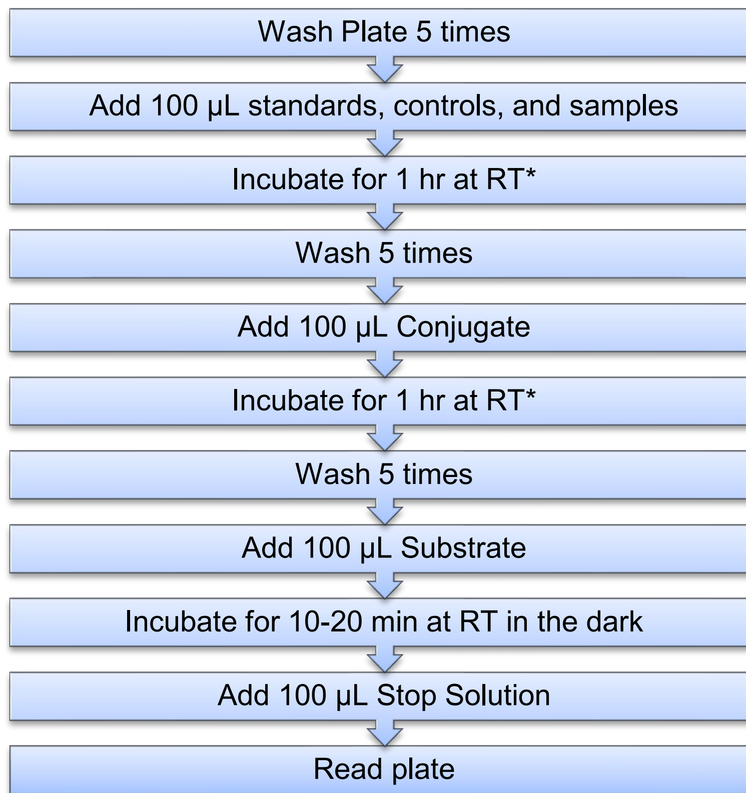
TECHNICAL HINTS

- Do not interchange different lot numbers of any kit component within the same assay. Furthermore, it is not recommended to assemble 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 colorless 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.

GENERAL NOTES ON THE ASSAY AND ASSAY PROCEDURE

- 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. ALPCO can therefore not be held responsible for any damage resulting from incorrect use.

SHORT ASSAY PROTOCOL



Total Time = 2 hours 20 minutes
***Set Plate shaker to 550 RPM, orbit 2mm**

SUGGESTED PLATE LAYOUT

Below is a suggested plate layout for running standards, controls, and up to 41 samples in duplicate.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std 1	Std 1	2	2	10	10	18	18	26	26	34	34
B	Std 2	Std 2	3	3	11	11	19	19	27	27	35	35
C	Std 3	Std 3	4	4	12	12	20	20	28	28	36	36
D	Std 4	Std 4	5	5	13	13	21	21	29	29	37	37
E	Std 5	Std 5	6	6	14	14	22	22	30	30	38	38
F	Ctrl 1	Ctrl 1	7	7	15	15	23	23	31	31	39	39
G	Ctrl 2	Ctrl 2	8	8	16	16	24	24	32	32	40	40
H	1	1	9	9	17	17	25	25	33	33	41	41

Std= Standard
Ctrl = Control
Numbered wells = Samples