ALBUWELL™ Hu ELISA

DESCRIPTION

Intended Use

Albuwell™ Hu is an enzyme linked immunosorbent assay (ELISA) for the quantitative determination of albumin in human urine. For Research Use Only. Not for use in diagnostic procedures.

Technical Background

Albuwell Hu is a tool for assessing the amount of albumin in human urine. It is a competitive antibody capture ELISA completed in a direct mode (see Appendix, Fig. 1). The anti-human albumin antibody is conjugated to horseradish peroxidase (HRP), i.e., directly labeled.

To complete the assay, sample and Anti-human Albumin Ab-HRP are added to human albumin-coated well of the microtiter plate. The antibody interacts and binds with the albumin immobilized to the stationary phase or with that in the fluid phase, hence the notion of competitive binding. Washing removes unbound Anti-human Albumin Ab-HRP and unbound reactants of the fluid phase from the well. Only the Anti-human Albumin Ab-HRP that was bound to the albumin of the stationary phase remains, and this is detected using tetramethylbenzidine (TMB) in a chromogenic reaction. The reaction is stopped with acid, and absorbance is measured at 450 nm. Absorbance is inversely proportional to the logarithm of albumin concentration in the fluid phase.

SPECIMEN COLLECTION AND STORAGE

1. Collect samples without preservative.
2. Clarify samples by centrifugation if necessary.
3. Store clarified urine at 4˚C for up to 1 week or at -80˚C for up to 2 months.

MATERIALS NEEDED

Kit Contents

- 2 Albuwell Assay Plates
- 2 NHEBSA (Diluent)
- Human Serum Albumin (hSA) Standard
- 2 Anti-human Albumin Ab-HRP Conjugate
- 2 Color Developer
- 2 Color Stopper
- Instructions

Note: hSA Standard, NHEBSA, Anti-human Albumin Ab-HRP Conjugate preparations contain 0.05% ProClin® 300 (active components isothiazolones) as preservative. Color Stopper contains dilute (2.0 N) sulfuric acid.

Albuwell Hu plates are pre-coated and ready-to-use. All kit reagents are supplied in ready to use liquid form. A plate wash procedure using tap water has been shown to be suitable in experimental and quality control contexts. However, where tap water is unavailable or unsuitable, we recommend using the Ethos Dilurex™ TEA Wash Buffer (pH 6.8) available at 10x and 1x concentrations.

Micropipettors capable of delivering 10, 50, 100, 120, and 200 µL are required. Multi-channel pipettors capable of delivering 50 and 100 µL are recommended. In addition, small test tubes are required to complete dilutions (microfuge tubes work well in this application). Finally, a microplate reader equipped to determine absorbance at 450 nm is required.

ASSAY PROCEDURE

Sample Preparation

1. Allow reagents and samples to come to room temperature before running the assay.
2. Do not apply heat to thaw samples.
3. For previously frozen samples:
    a. Vortex gently after fully thawed.
    b. Leave samples at room temperature for 1 hour to allow cryoprecipitates to settle out of the fluid column.

Standard Dilutions

This procedure describes the preparation of seven (7) two-fold dilutions of standard (see Appendix, Fig. 2).

1. Prepare 8 microfuge tubes with 200 µL of NHEBSA per tube.
2. Label the tubes C and 1 to 7.
3. Transfer 200 µL of hSA Standard to tube 1.
4. Mix contents thoroughly by gentle vortexing.
5. Transfer 200 µL of solution from tube 1 to tube 2.
6. Mix as before.
7. Continue this procedure through tube 7.
8. Tubes 1-7 now contain concentrations of 10.0, 5.0, 2.5, 1.25, 0.625, 0.313 and 0.156 µg hSA/mL respectively.

Preparation of Urine Sample Dilutions
Accurate determination of urinary albumin depends upon proper sample dilution. In most cases, a 1:21 dilution is sufficient, but collection time and/or kidney function (or dysfunction) may lead to exceptionally high or exceptionally low concentrations. For initial studies it is wise to complete the analysis at three concentrations. The results obtained will allow the choice of the best (single) dilution for subsequent analyses.

The following example illustrates a 1:21 dilution protocol:
1. Label a microfuge tube for each sample.
2. Add 200 µL NHEBSA to each tube.
3. Use a dry fresh tip to transfer 10 µL of sample to the appropriate tube.
4. Vortex the tube briefly.
5. Continue this procedure for the rest of the samples.
6. Each sample is now diluted 1:21 in NHEBSA.

Addition of Controls, Standard hSA Dilutions, and Samples to the Plate
Number the column strips of the 96-well plate with an indelible marker. This will allow reconstruction of the plate should strips fall out during the washing procedures. The diluted standards and samples maybe added directly to the dry plate. A plate map is provided to assist with this section, see Appendix, Fig. 3.

The plate design includes two controls. Well A1 is a negative control, and well A2 is a positive control. All other wells in column 1 and 2 are diluted hSA standards. The rest of the plate is available for diluted samples.

1. Add an 100 µL aliquot of NHEBSA from tube C to well A1. This will be the negative control. Do NOT add any Anti-human Albumin Ab-HRP conjugate during the primary incubation.
2. Add 50 µL of NHEBSA to well A2. This will be the positive control.
3. With a fresh tip, pre-wet the tip in standard dilution number 7, and transfer 50 µL aliqouts to wells H1 and H2.
4. With a new tip, pre-wet/rinse the tip in standard dilution number 6, and transfer 50 µL aliquots to wells G1 and G2.
5. Continue transferring diluted standard to the plate in this fashion, i.e. pre-wetting/rinsing a new tip for each dilution and transferring the aliquot to the appropriate well.
6. Taking care to change the tip and to pre-wet it each time, add diluted experimental samples to the plate. Samples can be added in duplicate starting at well A3 and A4 and continuing through the rest of the plate.
7. The plate now contains controls, standard dilutions, and sample dilutions.

Primary Incubation: Reaction with Anti-human Albumin Ab-HRP.
1. Skip well A1. This is the negative control.
2. Starting with well A2, add 50 µL of Anti-human Albumin Ab-HRP conjugate to the remaining wells.
3. Cover and incubate the plate for 30 mins.
4. Wash plate using a plate washer a total of 10 times.
5. Alternatively, plates can be washed manually using the following:
   a. Remove fluids from the well by aspiration or by flipping plate over a sink.
   b. Fill wells to overflowing with water or wash buffer.
   c. Remove fluids as before.
   d. Repeat the wash cycle a total of 10 times.
6. Invert the plate on a paper towel, tapping gently to remove excess fluids.

Color Development and Measurement
1. Add 100 µL of Color Developer to each well.
2. Incubate 5-10 mins to allow color to develop.
3. Add 100 µL of Color Stopper to each well.
4. Examine the plate. The negative control in well A1 should have little to no color. The positive control in well A2 should be the most intensely colored well on the plate. The rest of the wells should show absorbances intermediate between these extremes.
5. Use a plate reader to determine the absorbance at 450 nm. Use well A1 as the blank.

ANALYSIS

If using modeling software, this kit has enough dilutions to use the 5PL non-linear regression model. If the data is symmetrical enough, the data can degenerate into a 4PL model. If no modeling software is available, the following procedure can be used in MS Excel®.

1. Prepare a spreadsheet entering appropriate data including standard concentration, sample dilution, and absorbance data. Determine the mean for replicate wells.
2. Prepare a semi-logarithmic plot of standard dilutions with the log10 [hSA] on the x-axis and mean absorbance on the y-axis. This is the standard curve.
3. The data that fall into the linear portion of the standard curve constitute the usable portion of the assay.
4. Subject these data to semi-logarithmic analysis to yield a mathematical model, of the form: log10[hSA] = m A450 + b
5. hSA concentration is determined by taking the anti-log of the calculated values from this equation.
6. Multiply by 21 (or inverse dilution factor) to correct for the dilution.

QUALITY CONTROL

Record Keeping: It is good laboratory practice to record the lot numbers and dates for the kit components and reagents used for each assay.

LIMITATIONS

- Samples must not contain inhibitors for HRP, i.e. sodium azide. These will affect results.
- It is the responsibility of the investigator to determine if the presence of experimental compounds or their metabolites in the urine will affect the assay results.
- Gross microbiological contamination may affect assay results.
- Bloody urine specimens are unsuitable for use, even if clarified by centrifugation, since blood flow is a sign of contamination and since albumin concentrations in the blood are approximately 2000 times those normally found in urine.
- Semen contains significant levels of albumin and is also a potential source of contamination.

TROUBLE SHOOTING

1. No color appears after adding Color Developer: One or more reagents may have been adversely affected by storage above 8°C. One or more reagents may not have been added. Repeat assay. Be sure to store the kit appropriately.
2. Color in wells too light: Longer incubation with Color Developer may be required. If the color is still too light after 10 minutes development, repeat the assay but increase the primary incubation with Anti-human Albumin Ab-HRP conjugate to 1 hour.
3. Color in wells is too dark: Decrease the development time. If a 5-minute development is still too dark, repeat the assay and reduce the initial incubation with conjugate to 15 minutes.
4. If color is dark and the standard dilutions fail to show the appropriate concentration-dependent-response, Color Developer may have been contaminated with conjugate or the plate was poorly washed. Repeat the assay and take care in the pipetting and in the washing operations.
5. Color in sample well(s) is darker or lighter than lowest or highest concentrations of the standard curve. Change sample dilution protocol appropriately.
6. Poor agreement between duplicate wells: This is almost always due to pipetting error. Repeat the assay.
7. Microplate ELISAs may be prone to edge effects wherein the outer rows and columns show a darker response than the inner ones. This effect may be minimized by incubating the plate in a closed humid container. A plastic food storage container with a tight-fitting lid and a water moistened paper towel work well in this respect. Place the moistened towel in the bottom of the container and place the plate upon it. Position the cover and incubate as described.

CREATININE COMPANION ASSAY

The Creatinine Assay is designed for companion use with Exocell's albumin ELISAs, allowing expression of results as µg protein (i.e. albumin) per mg creatinine in the urine. This is typically used as a normalized value to compare samples.

For research use only. Not for use in diagnostic procedures. Purchaser is solely responsible for all decisions regarding the use of these products and any associated regulatory or legal obligations.

PRODUCT INFORMATION

CAT. #	DESCRIPTION
1004	Albuwell™ Hu ELISA
1012	Creatinine Companion Assay

Order today at **EthosBiosciences.com**

TRADEMARKS

APPENDIX

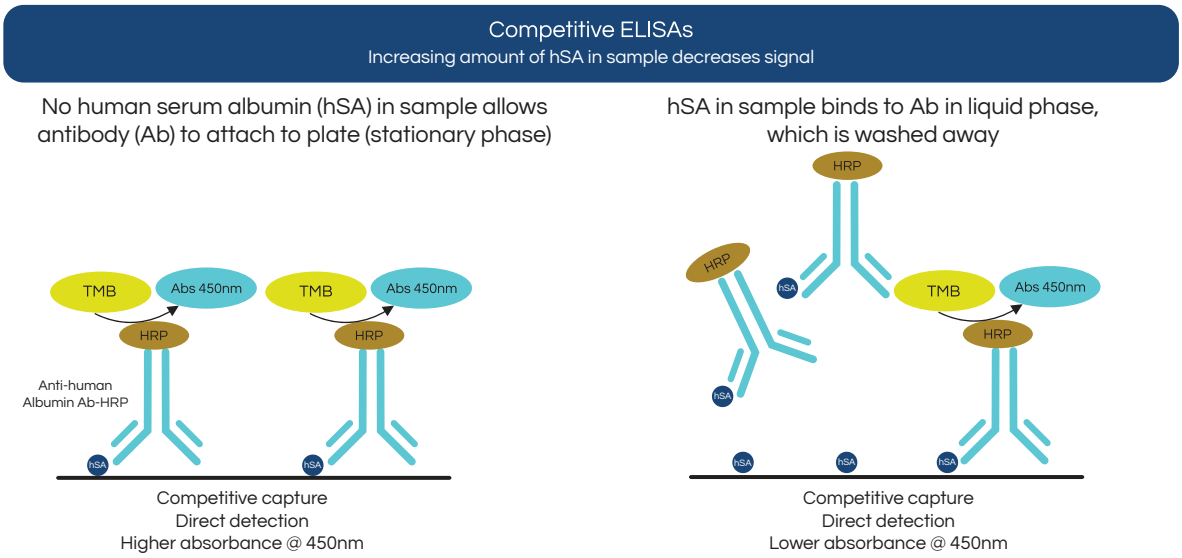
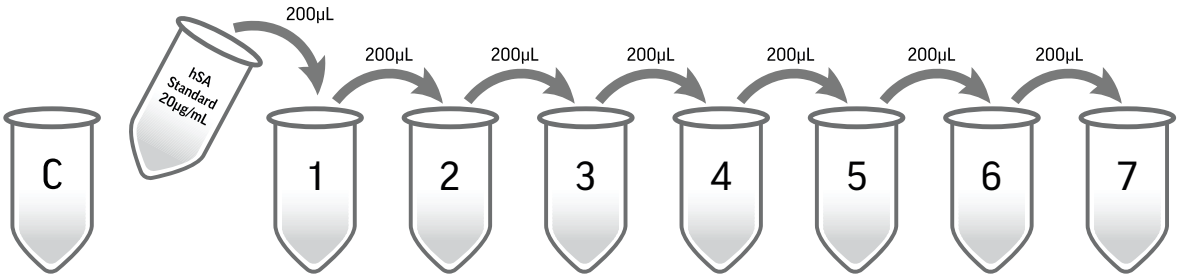


Fig. 1. Competitive ELISAs have decreasing signals when albumin is present in samples. The HRP molecule is directly attached to the Fc region of the anti-human albumin antibody, thus eliminating the need for adding a labeled secondary antibody. The left diagram represents what happens in well A2 of this kit. The right diagram represents the addition of hSA standards in wells B1 and B2 through H1 and H2. The more albumin in the sample, the lower the absorbance reading since the Anti-human Albumin Ab-HRP conjugate will wash away in the liquid phase rather than binding to the plate.



NHEBSA (µL)	200	200	200	200	200	200	200	200
Human Serum Albumin (hSA)	0	200 µL of stock	200 µL of 1	200 µL of 2	200 µL of 3	200 µL of 4	200 µL of 5	200 µL of 6
Final conc. of hSA (µg/mL)	0	10	5	2.5	1.25	0.625	0.313	0.156

Fig. 2. Creating the human serum albumin (hSA) standard dilution series. hSA is supplied at 20 µg/mL stock solution that is diluted at 1:2 seven consecutive times to establish a standard curve for estimating the amount of albumin in experimental samples.

APPENDIX, CONTINUED

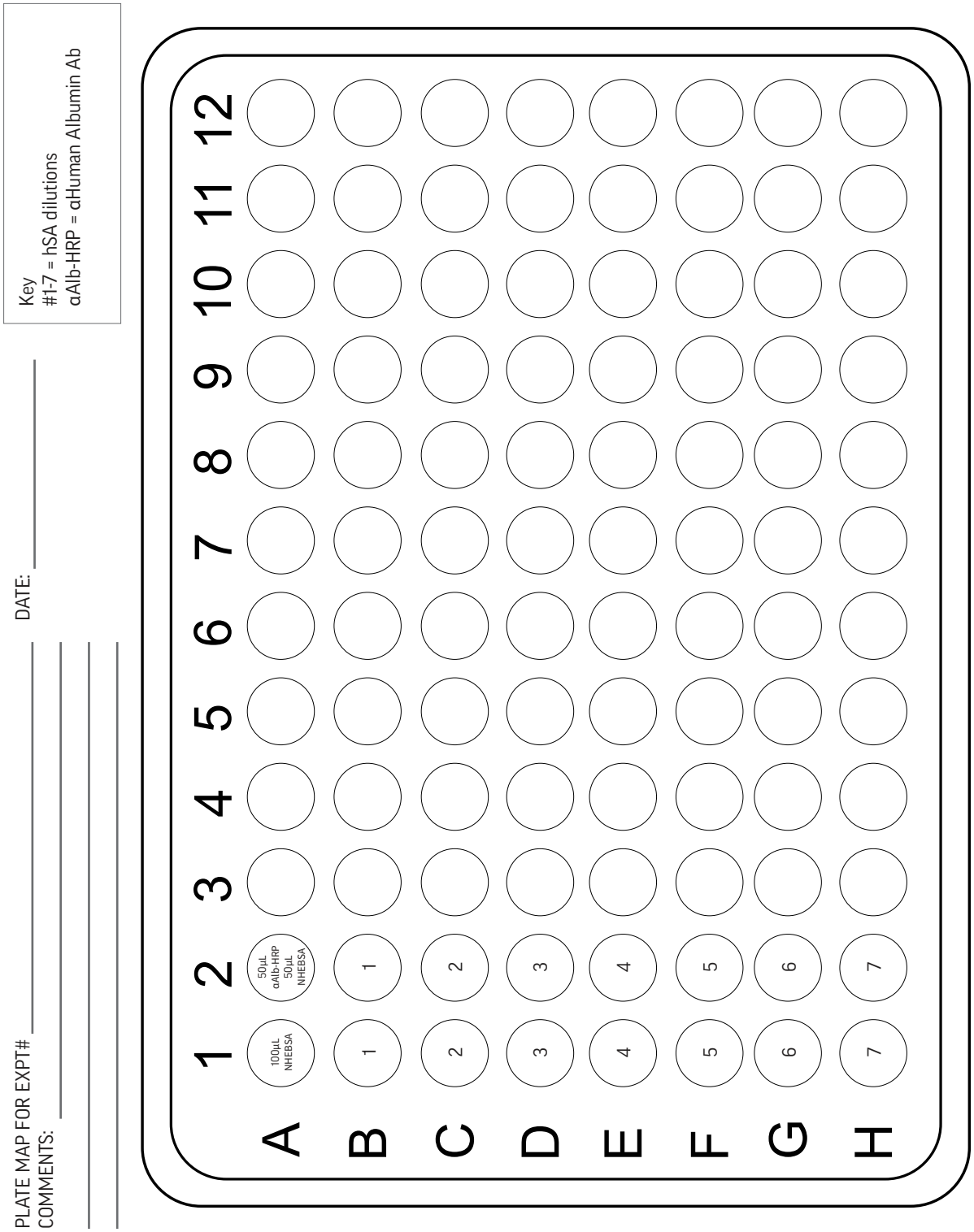


Fig. 3. Plate map. Well A1 has 100 µL of NHEBSA buffer only which will be the negative control. Well A2 has 50 µL of NHEBSA buffer + 50 µL of αHuman Albumin Antibody-HRP conjugate as a positive control. The remaining wells in column 1 and 2 have 50 µL of each hSA serial dilution in duplicate to create the standard curve. Columns 3 through 12 are available for up to 40 samples tested in duplicate.