ALBUWELL O

Dilute Standards and Samples

Add to Wells

Add Anti Albumin Antibody

Incubate for 30 Minutes

Add Conjugate

Incubate for 30 Minutes

Wash Plate

Develop for 5-10 Minutes

Add Color Stopper

Less than 1 ½ hours to complete

Exocell
1880 JFK Blvd.
Suite 200
Philadelphia, PA 19103

Phone: (215)557-8021
Fax: (215)557-8053
email:exocell@verizon.net
http://www.exocell.com

Albuwell O: An ovine microalbuminuria ELISA.

Intended Use: Albuwell O is an enzyme linked immunosorbent assay (ELISA) for the quantitative determination of albumin in sheep urine. It is for research purposes, and is not intended for diagnostic use.

Technical Background: Albuwell O is for in vitro use as a research tool for assessing kidney function in sheep. It is simple to perform and highly specific for sheep albumin. Albuwell O is a conventional indirect competitive ELISA that recognizes antigen (albumin) in test samples.

To complete the assay, sample and rabbit anti-ovine albumin antibody are added to an albumin-coated well. 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. A subsequent reaction with anti-rabbit -HRP conjugate labels the probe with enzyme. After washing, only the antibody-conjugate that bound to the albumin of the stationary phase remains in the well, and this is detected using a chromogenic reaction. Color intensity in Albuwell O is inversely porportional to the logarithm of albumin in the fluid phase.

Specimen Collection and Storage: Collect samples without preservative, and render them particulate free by centrifugation. Store clarified urine at 4^{o}C for up to 1 week or at -60^{o}C for up to 2 months. Prior to assay, allow the samples to come to room temperature. Do not apply heat to thaw frozen samples. Clarify samples by centrifugation if necessary.

Kit Contents: Your Albuwell O kit should contain the following items:

a. 2 Albuwell O Test Plates
b. 2 NHE-BSA Diluent
c. 2 Ovine Serum Albumin Standard
d. 2 Rabbit Anti-ovine Albumin Antibody
e. 2 Anti-rabbit HRP Conjugate
f. 2 Color Developer
g. 2 Color Stopper
h. Instructions

Ovine Serum Albumin (OSA) Standard, NHE-BSA Diluent, Rabbit anti-ovine Albumin Antibody and Anti-rabbit HRP Conjugate preparations contain 0.05% Proclin 300 (active components isothiazolones) as preservative. Color Stopper contains dilute (2.0 N) sulfuric acid.

Albuwell O plates are precoated and ready to use. All kit reagents are supplied in ready to use liquid form. A provision to wash the plates should be made, an EIA Wash Buffer with composition: 0.15 M NaCl, 0.01 M triethanolamine (pH 6.8), 0.05% Tween 20 and 0.05 % Proclin 300 (preservative may be omitted if the buffer is freshly prepared) is recommended.

Micropipettors capable of delivering 10, 50, 100 and 120 uL are required. Multi-channel pipettors capable of delivering 50 and 100 uL 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: Allow reagents and samples to come to room temperature before running the assay.

Standard Dilutions: The standard is supplied ready to use (100 ug/ml). This procedure describes the preparation of 6 two-fold dilutions of standard.

1. Prepare 6 microfuge tubes with 120 uL of NHE-BSA Diluent per tube.
2. Label the tubes1-6.
3. Transfer 120 uL of OSA Standard to tube 1.
4. Mix contents by aspirating and expelling the fluids 5 times.
5. Transfer 120 uL of solution from tube 1 to tube 2.
6. Mix as before.
7. Continue this procedure through tube 6.
8. Tubes 1-6 now contain dilutions of 50.0, 25.0, 12.5, 6.25, 3.125, and 1.56 µg/mL respectively.

Preparation of Urine Sample Dilutions: Accurate determination of urinary albumin depends upon proper sample dilution. In most cases, a 1:13 dilution is sufficient, but collection methods and animal 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, i. e. undilute, 1:20 and 1:80. The results obtained will allow the choice of a single dilution for subsequent analyses.

The following example illustrates a 1:13 dilution protocol.

1. Prepare and label a microfuge tube for each sample.
2. Add 120 uL NHE-BSA Diluent to each tube.
3. Use a dry fresh tip to transfer 10 uL of sample to the appropriate tube, wash out the tip by repeated aspiration and expellation.
4. Vortex the tube briefly.
5. Continue this procedure for the rest of the samples.
6. Each sample is now diluted 1:13 in NHE-BSA Diluent.

Addition of Controls, Standards and Samples to the plate: Label the strips with an indelible marker, 1-12. This will allow

reconstruction of the plate if strips fall out during the washing procedures.

1. Add 100 uL of NHE-BSA Diluent from the stock bottle to well A1. This is the negative control "C0" and will be used to standardize or "blank" the microplate reader.
2. Add 50 uL of NHE-BSA Diluent to well A2. This is the positive control "C1" and is a qualitative indicator of assay performance.
3. With a fresh tip, transfer 50 uL aliquots of Tube 6 dilution to wells H1 and H2.
4. With the same tip, transfer 50 uL aliquots from tube 5 to wells G1 and G2.
5. With the same tip, transfer 50 uL aliquots from tube 4 to wells F1 and F2.
6. With the same tip, transfer 50 uL aliquots from tube 3 to wells E1 and E2.
7. With the same tip, transfer 50 uL aliquots from tube 2 to wells D1 and D2.
8. With the same tip, transfer 50 uL aliquots from tube 1 to wells C1 and C2.
9. With a fresh tip, transfer 50 ul aliquots of the stock ovine albumin standard to wells B1 and B2.
10. In a similar manner, add 50 uL aliquots of diluted sample to wells A3 and A4.
11. Continue adding diluted samples to the plate.
12. The plate now contains controls and standard dilutions in wells A-H, 1,2, and diluted experimental samples in duplicate in the balance of the plate.

Primary Incubation: Reaction with Rabbit Anti-ovine Albumin Antibody

1. Add 50 uL of Rabbit Anti-ovine Albumin Antibody to Wells A2-A12, and B-H 1-12.
2. Incubate the plate covered for 30 minutes at room temperature.

Secondary Incubation: Reaction with Anti-rabbit HRP Conjugate:

1. Use a plate washer or wash plates by hand as follows:
   a. Remove fluids from the well, ie. aspirate off fluids or flip them out into a sink.
   b. Fill wells to over-flowing with recommended wash buffer.
   c. Remove fluids as before.
   d. "b" and "c" constitute a wash cycle.
   e. Repeat the process to yield a total of 6 wash cycles.
   f. Invert the plate on a paper towel and tap gently to remove adherent fluids.
2. Add 100 uL of Anti-rabbit HRP Conjugate to every well on the plate.
3. Incubate as before for 30 minutes.

Color Development:

1. Wash plate as above for 8 washes.
2. Add 100 uL of Color Developer to each well.
3. Develop 5- 10 minutes
4. Add 100 uL of Color Stopper to each well.

Analysis: Examine the plate. The negative control well, C0 which is in well A1, should have little or no color, but the positive control well, C1 which is in well A2, should be the most intensly colored well on the plate. The rest of the wells should show absrbances intermediate between these extremes.

This analysis assumes that computer and analysis software, e.g. MS Excel, is available.

1. Use a plate reader to determine the absorbances at 450 nm, use the C0 well in A1 to “blank” the reader.
2. Prepare a spreadsheet entering appropriate data including standard dilution, concentration, sample dilution and absorbance data. Determine the mean for replicate wells.
3. Prepare a semi-logarithmic plot of standard dilutions with the log [OSA] on the x-axis and mean absorbance on the y-axis. This is the dose-response or standard curve.
4. The data that fall into the linear portion of the dose-response curve constitute the usable portion of the assay.
5. Subject these data to semi-logarithmic analysis to yield a mathematical model, of the form

$$\log_{10} [\ OSA\ ] = m\ A_{450} + b$$

6. OSA concentration is determined by taking the anti-log of the calculated values from this equation.
7. Multiply by 13 (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 of the kit components and reagents for each assay.

Sample Handling: The samples should be secured, processed and stored as discussed above. Urine is often comtaminated by food and fecal material, and these contaminants present potential sources of error. Therefore, clarify the samples by centrifugation as required.

Dilute Standard and Samples carefully. For the standards, a single tip may be used to prepare the dilution series. For the experimental samples, a fresh tip should be used for each urine specimen. The tip should be used dry, hence not prewetted by sample, and washed out in the NHE-BSA Diluent by repeated aspiration and expellation.

Limitations:

1. 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.
2. Gross microbiological contamination may affect assay results.
3. 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 adversly affected by storage above 8^{o}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 and secondary incubations to 1 hour each.
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 secondary incubation to 15 minutes.

   If color is dark and the standard dilutions fail to show the appropriate dose-response, Color Developer may have been contaiminated with conjugate or the plate was poorly washed. Repeat the assay and take care in the pipetting and in the washing operations.
4. Color in sample well(s) is darker or lighter than lowest or highest concentrations of the standard curve. Change sample dilution protocol appropriately.
5. Poor agreement between duplicate wells: This is almost always due to pipetting error. Repeat the assay.

   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.

ver. 3.0 Ferbruary 2017