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Protocol for Albuwell M kit: Murine Microalbuminuria ELISA By Exocell Inc

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We use this protocol and it's working

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Abstract

Summary:

Albuwell M is an indirect competitive ELISA designed to monitor kidney function in the mouse by measurement of urinary albumin. To complete the assay, sample and rabbit anti-murine albumin antibody are added to albumin coated wells. The antibody interacts and binds with the albumin immobilized to the stationary phase or with albumin 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 bound to the stationary phase remains in the well, and this is detected using a chromogenic reaction. Color intensity is inversely proportional to the logarithm of albumin in the fluid phase. The assay may be completed in less than 2.5 hours

Diabetic Complication:

Nephropathy

Materials

MATERIALS

Albuwell M Kit **Exocell, Inc Catalog #**1011

Kit Contents:
2 Albuwell M test plates
2 EIA Diluent
1 Murine Serum Albumin (MSA) Standard
2 Rabbit Anti-Murine Albumin Antibody
2 Anti-Rabbit HRP Conjugate
2 Color Developer
2 Color Stopper

MSA Standard, EIA Diluent, Rabbit anti-murine Albumin Antibody, and Anti-rabbit HRP conjugate preparations contain 0.05 % Proclin 300 (active components isothiazolones) as preservatives. Color Stopper contains dilute (2.0 N) sulfuric acid. Save all unused reagents for future assays.

Albuwell M 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, tap water may used, but an EIA Wash Buffer with the 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 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.

Troubleshooting

Urine Collection Protocols

1 ***Spot Urine***

Between 8:00 and 10:00 a.m., the mouse is picked up and induced to urinate into a 1.5 ml microfuge tube. If this is not successful, or not enough is collected, attempts on successive days can be combined (urine should be stored at 4ºC between attempts).

Another way to collect spot urine is to place the mouse in a special cage (no food or water) the size of a 96-well plate. I have a custom made apparatus with compartments for eight mice. The mice stand on a 96-well plate for 2 hours. Urine is collected from the various wells the mouse urinates in, taking care to avoid any urine contaminated by feces.

2 ***24-hour***

Mice are weighed before placement into Techniplast 24-hr urine collection cages. Mice remain in the cages for 72 hours. The urine is collected and mice are weighed after each 24 hour period. Urine is stored at 4ºC until analysis. Food and water are replenished as necessary. The sample from the third 24-hour period is the preferred sample for albumin and creatinine measurement.

In general, if the sample is stored at 4ºC, then analysis will occur within 7 days. If longer stored is required, the sample can be frozen at -60ºC for two months.

3 ***Standard dilutions:***

This procedure is written to allow the construction of duplicate standard curves.

1. Prepare 7 microfuge tubes with 120 ul of EIA diluent per tube.

2. Label the tubes, C1, 1-6.

3. Transfer 120 ul of MSA 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 10.0, 5.0, 2.5, 0.625, and 0.313 ug/ml respectively.

4 ***Preparation of Urine Samples 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. undiluted, 1:20, 1:80. The results obtained will allow the choice of the best (single) dilution for subsequent analyses.

The following example illustrates a 1:13 dilution protocol.

1. Prepare and label microfuge tube for each sample.

2. Add 120 ul EIA 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 in the tube.

4. Vortex the tube briefly.

5. Continue this procedure for the rest of the samples.

6. Each sample is now diluted 1:13 in EIA Diluent.

5 ***Addition of Controls, Standard MSA Dilutions and Samples to the Plate:***

1. Add 100 ul aliquots of EIA Diluent from the stock bottle to wells A1 and A2. These are the negative controls “C0” and will be used to standardize or “blank” the microplate reader.

2. Add 50 ul aliquots from C1 tube to wells B1 and B2. These are the positive controls “C1” and are qualitative indicators 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. Continue adding standard dilution in this fashion.

6. In a similar manner, add 50 ul aliquots of diluted sample to wells A3 and A4.

7. Continue adding diluted samples to the plate.

8. 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.

6 ***Primary Incubation: Reaction with Rabbit Anti-murine Albumin Antibody***

1. Add 50 ul of Rabbit Anti-murine Albumin to wells A3-A12, and B-H 1-12.

2. Incubate the plate covered for 30 minutes at room temperature.

7 ***Secondary Incubation: Reaction with Anti-rabbit HRP Conjugate***

1. Use a plate washer or wash plates by hands as follows:
a. Remove fluids from well, ie. Aspirate off fluids or flip them out into the sink.
b. Fill wells to over-flowing with water or 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 10 wash cycles.
f. Invert 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.

8 ***Color Development:***

1. Wash plate as above.

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.

9 ***Analysis:***

1. Determine the Absorbance of experimental wells at 450 nm blanked against well A1.

2. Calculate the average absorbances and enter the values in your worksheet.

3. Prepare a semi-logarithmic plot of standard dilutions with the log [MSA] vs absorbance.

4. The data that fall into linear portion of the dose-response curve constitute the usable portion of the assay.

5. Subject these data to the semi-logarithmic analysis to yield a mathematical model, of the form $\log_{10}$ [MSA]=mA_{450}+b

6. Determine the estimated [MSA] for each experimental sample.

7. Multiply by 13 to correct for the dilution.

10 ***Quality Control:***

Record keeping: it is good laboratory practice to record the lot numbers and dates of the kit components and reagents of each assay.

11 ***Sample Handling:***

The samples should be secured, processed and stored as discussed above. Mouse urine is often contaminated by food and fecal material, and these contaminants present potential sources of error. Centrifugation to clarify samples is recommended.

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 dry, hence not prewetted by sample, and washed out in the EIA Diluent by repeated aspirations and expellation.

Limitations:

12 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.