Micro-Albumin

Cat # 1026Z

For in vitro Research Use Only

NAME AND INTENDED USE
The Diagnostic Automation Inc. Micro-Albumin Quantitative Test is a solid phase enzyme-linked immunosorbant assay (ELISA). This test provides quantitative measurement of Micro-Albumin in human urine. (For Professional Use Only)

SUMMARY AND EXPLANATION OF TEST
The analytical determination of the protein albumin in urine is important because increased values indicate an increased risk of developing end-stage renal diseases and cardiovascular disease among people with diabetes\(^1,\ 2\). Also albumin in urine is a sensitive indicator of renal damage caused by exposure to nephrotoxic \(^3,\ 4,\ 5\). The most significant and well-documented of these abnormalities is a subtle increase in the urinary albumin excretion rate, known as Micro-Albuminuria. Microalbuminuria is not measurable by conventional techniques for detecting proteinuria. It is believed that microalbuminuria represents a reversible stage of renal dysfunction, whereas overt proteinuria reflects irreversible disease. Proteinuria typically appears about twenty years after onset of diabetes, whereas microalbuminuria can be detected within the first ten years. Microalbuminuria (30-150 ug/min) has been established as a marker predictive of subsequent development of diabetic nephropathy. Periodic monitoring (2-3 times/year) of urinary albumin levels in the diabetic patient is therefore recommended so that the initial escalation of renal damage can be detected and appropriate treatment regimens can be instituted. Radial immunodifussion, immunoturbidmetric, immunonephelometric method and RIA have been used for the albumin assay in urine. DAI Micro-Albumin Quantitative using microwell competitive ELISA method provides a convenient, sensitive and specific assay for albumin and free of interference from urine specimens.
PRINCIPAL OF THE ASSAY
DAI Micro-Albumin Quantitative test system is a solid phase enzyme-linked immunosorbant assay (ELISA). The wells are coated with specific albumin. The samples, standards and controls are incubated in the wells with anti-albumin enzyme conjugate. Enzyme conjugate and albumin in the urine sample complete binding with albumin antigens in the well. Unbound enzyme conjugate is washed off with water. The amount of bound peroxidase is inverted proportional to the concentration of the albumin present in the samples, standards and controls. Upon addition of the TMB substrate a color is developed after a short incubation period. The enzyme reaction is stopped and the intensity of the color measured with microwell reader at 450 nm. When high levels of patient albumin are present in patient’s urine, less enzyme conjugate is bound, hence less color development is observed.

WARNING AND PRECAUTION
1. DAI Micro-Albumin quantitative is designed for in vitro use only.
2. The components in this kit are intended for use as an integral unit. The components from different lots should not be mixed and used.
3. References contains human serum should be treated as potentially infectious. All human based products should be used appropriate precautions.

MATERIALS PROVIDED
1. Microwell Strips (96 wells): Specific Micro-Albumin coated wells. 8x12 strips.
2. Enzyme Conjugate (11mL): Anti-Micro-Albumin Antibodies conjugated to horseradish peroxidase.
3. Sample Diluent (11 mL) or zero standard. Phosphate buffered saline with stabilizer.
4. Reference Standard Set (0.5 mL/each): Human MICRO-ALBUMIN. References: 2.5, 5, 25, 50, 100 µg/mL. Human Albumin Standard in the phosphate buffered saline. The Standards are calibrated to 0, 2.5, 5, 25, 50 and 100µg/mL.
5. Positive Control (0.5 mL) values as indicated on the vial.
6. TMB Solution (11mL): Buffer solution containing hydrogen peroxide and TMB
7. Washing Buffer Concentrate (100X) (10 mL): Prepare working washing solution by adding 10 mL washing buffer concentrate into 990 mL distilled water.
8. Stop solution (11 mL): 2N HCl
9. Well holder for securing individual well.

MATERIALS REQUIRED BUT NOT PROVIDED
1. Microwell reader with wavelength at 450 nm.
2. Pipetor with tips for measuring 25 uL, 100 uL.
3. 1 L washing bottle.

STORAGE AND STABILITY
1. Store the kits at 2-8°C and keep microwells in a dry bag with desiccants.
2. Reagents are stable until expiration of the kit. TMB Solution should be colorless. If the solutions turn blue, it must be replaced. Do not expose these reagents to strong light during storage or usage.

SPECIMEN COLLECTION AND HANDLING
Collect a single void, timed fractional, overnight or 24-hour sample without preservative-recording the time, duration and total volume of the collection. Mix well, withdraw a small portion, and clear by
centrifugation or filtration. Prior to assay, allow the samples to come to room temperature. Do not thaw samples by applying heat. The following precautions should be observed when testing for urinary albumin:

1. Urinary albumin excretion is increased by physiological factors such as the erect posture, exercise and acute diuresis. It follows that the method of urine collection must be standardized when screening and monitoring patients. Urine samples should not be collected undue exertion nor after an acute fluid load. Reference ranges must specify the type of urine collection.
2. The possibility of contamination with menstrual or seminal fluid or due to urinary tract infection should be borne in mind.
3. The urine specimen are kept at room temperature for a few minutes or stored at 4-8°C for a few hours or three days at most before assay. Frozen samples with Tween 20 (1mL/L) can be stored up to six months.

**PREPARATION FOR ASSAY**
1. Bring all reagents and samples to room temperature (24± 3°C) and mix gently before beginning the test.
2. Have all reagents and samples ready before the start of the assay. Once the test has begun, it must be performed without any interruption to get the most reliable and consistent results.
3. Use new disposable tips for each specimen.

**ASSAY PROCEDURE**
1. Secure the desired number of coated wells in the holder. Mark data sheet with sample identification.
2. Dispense 10 µL of samples, Standards into the assigned wells.
3. Dispense 100 µL of Enzyme conjugate into each well and mix for 5 seconds.
4. Incubate for 30 minutes at Room Temp.
5. Remove incubation mixture and rinse the wells five times with Washing Buffer (300 µL/well/each rinse).
6. Dispense 100 µL of TMB Solution into each well including the blank well
7. Incubate for 15 minutes at R.T.
8. Stop reaction by adding 50 µL of Stop solution to each well and read O.D. at 450 nm with a microwell reader.

**PROCEDURALNOTE**
1. Wash the microwells and remove washing buffer thoroughly to get the Best results.
2. Pipet all reagents and samples into bottom of the well. Vortex-mixing of shaking is not required.
3. Absorbance is a function of the time and temperature of incubations. It is recommended to have reagents, samples and needed wells ready. Ensure the equal elapsed time for each pipetting ‘without interruption.
4. For the same reason the size of the of the assay run should be limited. It is suggested to run no more than 20 patient samples with a set of Reference Standards in duplicate.
5. If a serum specimen contains greater than 100 ug/mL or Micro-albumin, the sample must be diluted with sample diluent and reassayed as described in the assay procedure.

**CALCULATION OF RESULTS**
1. Plot the concentration (X) of each Reference Standards against its absorbance (Y) on the graph paper.
2. Obtain the values of patient samples by reference to the Standard curve. (following data is for demonstration purpose only)
### Well No. | Description (µg/mL) | Absorbance (450 nm) | Micro-Albumin (µg/mL)
---|---|---|---
A1 | 0 | 3.157 |
B1 | 2.5 | 2.505 |
C1 | 5 | 2.067 |
D1 | 25 | 0.458 |
E1 | 50 | 0.250 |
F1 | 100 | 0.109 |
G1 | Patient 1 | 1.222 | 9.7 |
H1 | Patient 2 | 0.696 | 16.9 |
A2 | Patient 3 | 0.555 | 20.8 |
B2 | Patient 4 | 0.212 | 56.9 |
C2 | Patient 5 | 0.175 | 76.0 |

### EXPECTED VALUES

1. It is recommended that each laboratory should determine its own normal and abnormal range.
2. Timed overnight samples and 24 hour samples have been commonly used to study microalbuminuria. The upper limits of urinary albumin excretion in healthy adults are approximately 26 mg/24 hour (18ug/min) and 9 ug/min in overnight samples.

Urinary albumin concentration in normal adults subjects:

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>First Daytime</td>
<td>6.72 mg/L (1.89-23.9)</td>
</tr>
<tr>
<td>Cumulated (24 hours)</td>
<td>5.33 mg/L (1.31-22.7)</td>
</tr>
</tbody>
</table>

3. In health subjects, albumin is ordinarily present in urine in the low range, with sustained values greater than about 15-30 mg/L usually being regarded as abnormal6.
4. Urinary albumin from 123 diabetics patients were reported with ranges 4.8-209 mg/L and mean values 46.8-61.4 mg/L4.
LIMITATIONS
1. For diagnostic purposes, the micro-albumin value should be used as an adjunct to other data available to the physician.
2. Samples with a pH of less than 4 or greater than 8 may yield results which are respectively too high or too low. Acidified samples are unsuitable for assay.
3. The assay should not be performed if the samples exhibit significant bacterial growth or if the patient shows signs of urinary tract infection.
4. Bloody specimens are unsuitable for use, even if clarified by centrifugation, since blood flow is a likely sign of contamination.

QUALITY CONTROL
Each laboratory should utilize internal controls at several levels to monitor assay performance. The controls should be treated as unknown. Values obtained should be in agreement with the assigned values of the control. Controls can be obtained from commercially available sources but should not contain sodium azide as preservatives.

PERFORMANCE CHARACTERISTICS
Accuracy
Recovery studies were performed by mixing an aliquot of diluted pooled urine sample and a albumin standard. The albumin values were measured and percentage of recovery were determined.

<table>
<thead>
<tr>
<th>Initial Values (ug/mL)</th>
<th>Expect Values (ug/mL)</th>
<th>Observd Values (ug/mL)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>15</td>
<td>17.0</td>
<td>113.3</td>
</tr>
<tr>
<td>5</td>
<td>27.5</td>
<td>29.5</td>
<td>107.3</td>
</tr>
<tr>
<td>10</td>
<td>17.5</td>
<td>18.3</td>
<td>104.6</td>
</tr>
<tr>
<td>10</td>
<td>30</td>
<td>30.8</td>
<td>102.7</td>
</tr>
<tr>
<td>20</td>
<td>22.5</td>
<td>23.7</td>
<td>105.3</td>
</tr>
<tr>
<td>20</td>
<td>35</td>
<td>36.2</td>
<td>103.4</td>
</tr>
</tbody>
</table>

Precision
Inter-assay and intra-assay co-efficient of variations were evaluated at three different pooled serum samples.

<table>
<thead>
<tr>
<th>Intra-assay</th>
<th>Pool A</th>
<th>Pool B</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Mean (ug/mL)</td>
<td>16.77</td>
<td>60.2</td>
</tr>
<tr>
<td>S.D. (ug/mL)</td>
<td>1.91</td>
<td>7.6</td>
</tr>
<tr>
<td>C.V. (%)</td>
<td>11.39</td>
<td>12.6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Inter-assay</th>
<th>Pool A</th>
<th>Pool B</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Mean (ug/mL)</td>
<td>16.3</td>
<td>55.8</td>
</tr>
<tr>
<td>S.D. (ug/mL)</td>
<td>2.18</td>
<td>3.46</td>
</tr>
<tr>
<td>C.V. (%)</td>
<td>13.4</td>
<td>6.19</td>
</tr>
</tbody>
</table>

Specificity
The addition of each of the following compounds to urine samples does not interfere with the measuring of albumin by using DAI Micro-Albumin Quantitative:
<table>
<thead>
<tr>
<th>Glucose</th>
<th>40 ug/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascorbic Acid</td>
<td>2 mg/mL</td>
</tr>
<tr>
<td>Transferrin</td>
<td>30 ug/mL</td>
</tr>
<tr>
<td>Creatinine</td>
<td>10 mg/mL</td>
</tr>
<tr>
<td>Uric Acid</td>
<td>1.5 mg/mL</td>
</tr>
<tr>
<td>Retinol Binding Protein</td>
<td>1 mg/mL</td>
</tr>
</tbody>
</table>

The antibody used in DAI Micro-Albumin Quantitative test is highly specific for human albumin. Cross reactivity with albumin from other species and human proteins other than albumin was not detected such as rat albumin, bovine albumin, chicken ovalbumin, rabbit albumin and human IgG.

**Sensitivity**

A linear study was performed to assess the sensitivity of the assay:

<table>
<thead>
<tr>
<th>Initial (ug/mL)</th>
<th>Dilution Folds</th>
<th>Expected (ug/mL)</th>
<th>Observed (ug/mL)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>1:2</td>
<td>50</td>
<td>52.8</td>
<td>105.6</td>
</tr>
<tr>
<td></td>
<td>1:3</td>
<td>33.3</td>
<td>34</td>
<td>102.1</td>
</tr>
<tr>
<td></td>
<td>1:4</td>
<td>25</td>
<td>24.1</td>
<td>96.5</td>
</tr>
<tr>
<td></td>
<td>1:5</td>
<td>20</td>
<td>18.7</td>
<td>93.4</td>
</tr>
<tr>
<td></td>
<td>1:10</td>
<td>10</td>
<td>11.0</td>
<td>110</td>
</tr>
<tr>
<td></td>
<td>1:20</td>
<td>5</td>
<td>6.63</td>
<td>133</td>
</tr>
</tbody>
</table>

The minimal detectable concentration of Micro-Albumin is estimated to be 1 ug/mL.

**REFERENCES**


<table>
<thead>
<tr>
<th>Date Adopted</th>
<th>Reference No.</th>
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<tbody>
<tr>
<td>2008-02-03</td>
<td>DA-Micro-Albumin-2008</td>
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