Application Note
General Guideline for Determination of Albumin in urine (Microalbuminurialy)

General information

Intended use
The Application Note is intended for the quantitative determination of albumin in human urine by instruments for which no specific guidelines exist (1). Performance on an instrument chosen by the customer should be validated.

Measuring range
Approximately 2-100 mg/L depending on the specific lot of the calibrator. In case of post-concentration or -dilution the range can be expanded.

Reference interval
0-42 mg/L (2). It is recommended to determine the reference interval for the local population.

Instrument settings
Guidelines for instrument programming are presented in “Suggestion for Instrument Settings...” on page 3.

Code No.   Name
Reagents
Antibody   Q0328 Dako Polyclonal Rabbit Anti-Human Albumin
Reaction buffer   S2007 Dako Reaction Buffer 1
Diluent   S2005 Dako Dilution Buffer 1
Calibrator   X0975 Dako Human Urine Protein Calibrator
Controls   X0976 Dako Human Urine Protein Low Control
            X0977 Dako Human Urine Protein High Control

Samples
Human urine.
In order to exclude samples with high concentrations of albumin, which might cause antigen excess and thus give erroneous results, samples should be checked with single use device for determination of albumin in urine (e.g. Albustix™).
Stable for 3 days at 2-8 ºC.
Stable for 3 months at –20 ºC (if only frozen once) (3).
Frozen samples should be thawed at 37 ºC and mixed well before analysis.

Calibrator
Dilution of standards is either performed automatically by the instrument as indicated or done manually.

Reaction buffer
The reaction buffer is ready for use. On board stability is 28 days at 2-12 ºC.

Antibody
Predilute the antibody in accordance with your final setting.
If in rare cases the prediluted antibody appears slightly turbid, filtration through a 0.22 µm membrane filter is recommended.
Stability of undiluted antibody: See expiry on the label.
Stability of prediluted antibody: 28 days at 2-8 ºC.
On board stability: 28 days at 2-12 ºC.
Capacity: 1 mL of prediluted antibody is equivalent to approximately 10 cuvette readings of standards or samples when 100 µL diluted antibody solution is used per test. The dead volume of the reagent bottle should be considered when calculating the required amount of reagents.

Calibration stability
It is recommended to recalibrate every 28th day or when reagent lots change, a new antibody dilution is prepared, the antibody dilution is filtered, or quality control results fall outside the range as established by the individual laboratory. However, the calibration stability should be validated on the individual instrument.

Trouble shooting
If performance is unacceptable, try to recalibrate. Check reagents and procedure. If the problem persists, please contact instrument supplier or Dako Technical Service.
**An Example of Performance Data on Hitachi 917**

**Sensitivity**
An OD value of approximately 0.46 on Hitachi 917 corresponds to an albumin concentration of approximately 200 mg/L.

**Detection limit**
The detection limit is estimated to 1.0 mg/L.

**Precision**
The precision was estimated by ANOVA analysis. Three samples with different albumin (UALB) levels were included in 6 runs each. Each sample was tested 6 times in each run and a new calibration was performed for each run.

<table>
<thead>
<tr>
<th>Sample</th>
<th>UALB Mean value (mg/L)</th>
<th>Standard deviation (mg/L)</th>
<th>Total CV (%)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Within run</td>
<td>Between run</td>
<td>Total</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>0.18</td>
<td>0.11</td>
<td>0.21</td>
</tr>
<tr>
<td>2</td>
<td>51</td>
<td>0.48</td>
<td>0.20</td>
<td>0.52</td>
</tr>
<tr>
<td>3</td>
<td>127</td>
<td>2.03</td>
<td>0.44</td>
<td>2.08</td>
</tr>
</tbody>
</table>

**Accuracy**
A recovery of albumin of 85-115% can be expected for Dako Human Urine Protein Low Control, code X0976 and Dako Human Urine Protein High Control, code X0977.

**Linearity**
The assay is linear in the range 2-900 mg/L.

**Security range**
No antigen excess is found for albumin concentrations up to 3000 mg/L.

**Interference**
No cross-reaction has been observed with known proteins.
No interference is found at concentrations up to 5 g/L of creatinine, 50 g/L of urea, 100 g/L of glucose, 25 mmol/L of calcium and 400 mg/L of alpha-1-microglobulin.
Samples containing hemoglobin may give a too high value of albumin in the low end of the measuring range.
All drugs described in (4) were investigated in urine samples according to the recommendations in (4). No interference was observed.

**Method comparison**
Determination of albumin in urine according to this Application Note was compared with other commercial turbidimetric assays. Data are available on request.

**References**
Suggestion for Instrument Settings for Human Serum Albumin in Urine

**Special Recommendations**  
A primary wavelength of approximately 415 nm should be used.

**Parameter** | **Suggestion**
--- | ---
Light path (mm) | 10
Incubation time (s) | 120
Sample dilution (Dil. factor) | 1
Volume of prediluted sample (µL) | 60
Sample volume (µL) (=Neat) | 60
Diluent+Flush (for Sample+Reag) (µL) | 0
Reaction buffer volume (µL) | 750
PEG-conc. in Reac. buffer (%) | 4
PEG-conc. in incubation volume (%) | 3.7
Incubation volume (µL) | 810
Antibody dilution (Ab dil. factor) | 6
Volume of diluted antibody (µL) | 150
Antibody volume (µL) (=Neat) | 25.0
Diluent+Flush (for Antibody) (µL) | 40
Total volume (µL) | 1000
Reaction time (s) | 300
Total analysis time (s) | 420

**Ratio**  
\[
\text{Sample vol. (Neat)} / \text{Antibody vol. (Neat)} = 2.4 \quad [2.4 - 2.6]
\]

**Relation**  
\[
\text{PEG - conc. in incub. vol. (%)} \times \text{Sample vol. (Neat) / Incub. vol.} = 0.27 \quad [0.26 - 0.29]
\]

**Ratio**  
\[
\text{Sample vol. (Neat)} / \text{Total vol.} = 0.060 \quad [0.058 - 0.062]
\]

**Standards**  

<table>
<thead>
<tr>
<th>Std</th>
<th>Dilution factor</th>
<th>f, Relative conc. factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Std 1 (Highest conc.)</td>
<td>100</td>
<td>0.010</td>
</tr>
<tr>
<td>Std 2</td>
<td>40</td>
<td>0.025</td>
</tr>
<tr>
<td>Std 3</td>
<td>15</td>
<td>0.067</td>
</tr>
<tr>
<td>Std 4</td>
<td>4</td>
<td>0.250</td>
</tr>
<tr>
<td>Std 5</td>
<td>2</td>
<td>0.500</td>
</tr>
<tr>
<td>Std 6 (Lowest conc.)</td>
<td>1</td>
<td>1.000</td>
</tr>
</tbody>
</table>

* For further explanations to the individual parameters please refer to "Introduction to Dako General Application Notes", order No. 30134.Intro, which is available on request or at www.Dako.com.

[ ] Indicates an acceptable range found on various instruments.

f \_i \_i \_i \_i is the relative concentration factor used to calculate the relative concentration (RC_{[i]}) of albumin in the standards from a specific lot of calibrator (C_{(Cal)}); RC_{[i]} = f_{[i]} \times C_{(Cal)} .