Application Note
Guideline for Determination of Prealbumin (Transthyretin) in Serum/Plasma on Modular P

General information

Intended use
The Application Note is intended for the quantitative determination of prealbumin in human sample material by turbidimetry on Modular P (1, 2).

Measuring range
Approximately 0.03-0.80 g/L depending on the specific lot of the calibrator. In case of post-concentration or -dilution the range can be expanded.

Reference interval
0.2-0.4 g/L (3). It is recommended to determine the reference interval for the local population.

Instrument settings
Instrument programming is performed according to "Instrument Settings" on page 3.

Reagents

<table>
<thead>
<tr>
<th>Code</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q0362</td>
<td>Dako Polyclonal Rabbit Anti-Human Prealbumin</td>
</tr>
<tr>
<td>S2007</td>
<td>Dako Reaction Buffer</td>
</tr>
<tr>
<td>S2005</td>
<td>Dako Dilution Buffer</td>
</tr>
<tr>
<td>X0908</td>
<td>Dako Human Serum Protein Calibrator</td>
</tr>
<tr>
<td>X0939</td>
<td>Dako Human Serum Protein Low Control</td>
</tr>
<tr>
<td>X0940</td>
<td>Dako Human Serum Protein High Control</td>
</tr>
</tbody>
</table>

Samples
Human serum, heparin-plasma or EDTA-plasma.
Stable for 7 days at 2-8 °C.
Stable for 3 months at –20 °C (if frozen only once) (4).
Frozen samples should preferably be thawed at 37 °C and mixed well before analysis.

Calibrator
Dilution of standards is performed automatically by the instrument.

Reaction buffer (R1)
The reaction buffer is ready for use. On board stability is 28 days at 2-12 °C.

Antibody (R3)
Predilute the antibody 1:4.40 (e.g. 4000 µL antibody + 13600 µL diluent).
If in rare cases the prediluted antibody appears slightly turbid, filtration through a 0.22 µm membrane filter is recommended.
Stability of undiluted antibody stored at 2-8 °C: See expiry on the label.
Stability of prediluted antibody: 28 days at 2-8 °C.
On board stability: 28 days at 2-12 °C.
Capacity: 1 mL of prediluted antibody is equivalent to approximately 18 cuvette readings of standards or samples. The dead volume of the reagent bottle should be added when calculating the required amount of reagent.

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

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.
Performance Data

Sensitivity
An OD value of approximately 0.15 on Modular P corresponds to a prealbumin concentration around 0.8 g/L.

Detection limit
The detection limit is estimated to 0.01 g/L.

Precision
The precision was estimated by testing at 4 different prealbumin (PREA) levels by ANOVA analysis of 6 runs each with a new calibration and 6 determinations in each run.

<table>
<thead>
<tr>
<th>Sera</th>
<th>PREA Mean value (g/L)</th>
<th>Standard deviation (g/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>0.11</td>
<td>0.003</td>
<td>0.002</td>
<td>0.003</td>
</tr>
<tr>
<td>2</td>
<td>0.24</td>
<td>0.004</td>
<td>0.002</td>
<td>0.004</td>
</tr>
<tr>
<td>3</td>
<td>0.27</td>
<td>0.005</td>
<td>0.001</td>
<td>0.005</td>
</tr>
<tr>
<td>3</td>
<td>0.65</td>
<td>0.007</td>
<td>0.001</td>
<td>0.007</td>
</tr>
</tbody>
</table>

Accuracy
A recovery of prealbumin of 90–110% can be expected for Dako Human Serum Protein Low Control, Code X0939, and Dako Human Serum Protein High Control, Code X0940.

Linearity
The assay is linear in the range 0.03-0.80 g/L.

Security range
No antigen excess is found for prealbumin concentrations up to 2.9 g/L (the highest concentration tested).

Interference
No interference is found at concentrations up to 10 g/L of hemoglobin, 600 mg/L of bilirubin, 25 g/L of triglyceride and 200 IU/mL of rheumatoid factor.
All drugs described in reference 5 were investigated according to the recommendations in reference 5. No interference was observed, except for intralipid at 10 g/L.

Method comparison
Determinations of prealbumin according to this Application Note was compared with other commercial turbidimetric assays. Data are available on request.

References
2. Modular P manual(s).
### Instrument Settings (Software Version 7675850-06-06)

#### Analysis

<table>
<thead>
<tr>
<th>Assay/Time/Point</th>
<th>2 Point End</th>
<th>10</th>
<th>15</th>
<th>34</th>
<th>0</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength (2nd/Primary)</td>
<td>700</td>
<td>340</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample Volume</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>24</td>
<td>10</td>
<td>86</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decrease</td>
<td>12</td>
<td>10</td>
<td>98</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increase</td>
<td>35</td>
<td>10</td>
<td>125</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reagent Volume</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R1</td>
<td>250</td>
<td>0</td>
<td>901*</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R2</td>
<td>0</td>
<td>0</td>
<td>901*</td>
<td>0</td>
<td>T2</td>
<td></td>
</tr>
<tr>
<td>R3</td>
<td>50</td>
<td>20</td>
<td>901*</td>
<td>0</td>
<td>T3</td>
<td></td>
</tr>
<tr>
<td>R4</td>
<td>0</td>
<td>0</td>
<td>901*</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### Diluent

- **Option:** Water
- **Diluent:**
  - Abs. Limit: 32000
  - Prozone Limit: 32000
  - Cell Detergent: Detergent 1
  - Twin Test: Cancel

#### Calibration

- **Calibration Type:** Logit-Log 4P
- **Auto Calibration**
  - Point: 6
  - Span: 4
  - Weight: 0
  - Update Type: None
  - Isozyme Q Channel: Cancel
- **SD Limit:** 50
- **Duplicate Limit:** 20%
- **S1 Abs. Limit:** 32000

#### Range

- **Application Code:** 901*
- **Report Name:**
  - Prealbumin
    - Male: 100 Year 99999 99999
    - Female: 100 Year 99999 99999
- **Data Mode:**
  - Active
  - Female: Std1+0.001 Std. 6
  - Male: Std. 1 Std. 6
- **Technical Limit:**
  - Std1: 0.001
  - Std. 6: 100 Year 99999 99999
- **Repeat Limit:**
  - Std. 1 Std. 6: 100 Year 99999 99999
- **Control Interval Time:**
  - Qualitative:
    - Sex:
      - Male
      - Female
    - Range:
      - Range 1
      - Range 2
      - Range 3

#### Others

- **Calibration Code:** 501*
- **Concentration:**
  - Std1: x C(cal)
  - Std. 6: x C(cal)
- **Rack No. - Pos.:**
  - S001-3*: S001-3*
- **Sample volume:**
  - Diluted S. Volume: 4
- **Diluent Volume:**
  - Diluted S. Volume: 11
- **Diluent – Code:** S2005
- **R1 – Code:** S2007
- **R3 – Code:** Q0362.

[*] Defined by the customer.
[**] The calculated concentration of Std.1 and Std.6. In order to get a warning for samples with concentration below the concentration of the lowest standard, the lower technical limit has to be 0.001 g/L higher than the lowest standard.
[***] Calibrator, Code X0908. The concentration is calculated as the factor times the calibrator value for the specific lot (stated in g/L on the X0908 Analytical Value Sheet). Number of decimals stated for Std.1 defines the number of decimals in printout. Two decimals are recommended.