Application Note
Guideline for Determination of Fibronectin in Serum on IMMAGE® Immunochemistry System

General information

Intended use
The Application Note is intended for the quantitative determination of Fibronectin in human serum by nephelometry on IMMAGE® Immunochemistry System (1, 2).

Measuring range
Approximately 0.03 – 0.75 g/L depending on the specific lot of the calibrator.

Reference interval
0.20-0.40 g/L (3). It is recommended to determine the reference interval for the local population.

Instrument settings
Instrument programming is performed according to the “Instrument Settings” on page 3.

Code
Name
Reagents
Antibody
Q0149
Polyclonal Rabbit Anti-Human Fibronectin
Reaction buffer
S2006
Dako Turbidimetry/Nephelometry Reaction Buffer 3
Calibrator
OUID
Dade Behring N Protein Standard PY
Control
OWSY
Dade Behring N/T Protein Control PY
Diluent
447640
IMMAGE® Immunochemistry System DIL 1

Samples
Human EDTA-plasma. Human serum has approximately 20% lower concentrations Stable for 7 days at 2-8 ºC. (3)
Samples are diluted with IMMAGE® Immunochemistry System DIL 1.

Calibrator
Dilution of calibrator is performed manually either in IMMAGE® Immunochemistry System DIL 1 or NaCl solution, 154 mmol/L (0.9%) or Dako Dilution Buffer 1.

Reaction buffer
The reaction buffer is ready for use.

Antibody
Predilute the antibody 1:1.70 (ex. 5000 µL antibody + 3500 µL diluents). If in rare cases the 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.
Capacity: 1 mL of diluted antibody is equivalent to approximately 44 cuvette readings of standards or samples. The dead volume of the reagent bottle should be considered when calculating the required amount of reagent.

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.

Trouble shooting
If performance is unacceptable, try to recalibrate. Check reagents and procedure. If the problem persists, please contact the instrument supplier or Dako Technical Service.

References
2. IMMAGE® Immunochemistry System Operation Manual (s). IMMAGE is a registered trademark of Beckman Coulter, Inc., Fullerton, CA
Performance Data

Sensitivity
Typical values found at 0.75 g/L Fibronectin are approximately 303 instrument responses on BeckmanImmage.

Detection limit
The detection limit is estimated to 0.015 g/L.

Precision
The precision was estimated on one control and two serum samples by ANOVA analysis of 6 runs each with a new calibration and 6 determinations in each run.

<table>
<thead>
<tr>
<th></th>
<th>Standard deviation g/L</th>
<th></th>
<th>Total CV (%)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fibronectin mean value</td>
<td>Within-run</td>
<td>Between run</td>
<td>Total CV (%)</td>
</tr>
<tr>
<td></td>
<td>g/L</td>
<td></td>
<td>g/L</td>
<td></td>
</tr>
<tr>
<td>Low human serum pool</td>
<td>0.081</td>
<td>0.002</td>
<td>0.011</td>
<td>0.011</td>
</tr>
<tr>
<td>N/T Protein Control PY</td>
<td>0.271</td>
<td>0.007</td>
<td>0.006</td>
<td>0.009</td>
</tr>
<tr>
<td>High human serum pool</td>
<td>0.293</td>
<td>0.007</td>
<td>0.008</td>
<td>0.011</td>
</tr>
</tbody>
</table>

Linearity
Linearity is acceptable in the range of 0.1-0.44 g/L, the highest concentration tested.

Security range
No antigen excess is found for Fibronectin concentrations < 3.1 g/L (highest concentration tested).

Interference
No interference is found at concentrations up to 10 g/L of hemoglobin, 420 mg/L of bilirubin, 2 g/L intralipid and 200 IU/mL of rheumatoid factor.
**Instrument Settings for IMAGEM® Immunochemistry System**

**Procedure**

1. Program a user-defined reagent with the parameters listed below. For more detailed instructions, please refer to Chapter 5 of the IMAGEM Immunochemistry System Operations Manual.
3. Transfer Dako Reaction Buffer 3 to compartment "B" of the cartridge. Please see the Note below.
4. Name the Chemistry “Frb”.
5. The calibrator value is lot dependent. When using the Dade Behring N Protein Standard PY (Code OUID) the following dilution series is prepared manually according to the dilution scheme shown below.
6. Use IMAGEM Dil1 (Beckman Coulter PN 447255) as the sample diluent and configure it on the IMAGEM as DIL1.

**Dilution schema**

<table>
<thead>
<tr>
<th>Standard</th>
<th>N Protein Standard PY</th>
<th>NaCl 0.9%</th>
<th>( f_{(i)} )</th>
<th>Conc.</th>
<th>( C_{(i)} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. (i)</td>
<td>( \mu L )</td>
<td>( \mu L )</td>
<td></td>
<td>Factor</td>
<td>Concentration</td>
</tr>
<tr>
<td>Std. (1)</td>
<td>[ 5 ]</td>
<td>[ 250 ]</td>
<td>( f_{(1)} )</td>
<td>0.0196</td>
<td>0.0196 x ( C_{(Cal)} )</td>
</tr>
<tr>
<td>Std. (2)</td>
<td>[ 8 ]</td>
<td>[ 105 ]</td>
<td>( f_{(2)} )</td>
<td>0.0707</td>
<td>0.0707 x ( C_{(Cal)} )</td>
</tr>
<tr>
<td>Std. (3)</td>
<td>[ 24 ]</td>
<td>[ 115 ]</td>
<td>( f_{(3)} )</td>
<td>0.1727</td>
<td>0.1727 x ( C_{(Cal)} )</td>
</tr>
<tr>
<td>Std. (4)</td>
<td>[ 40 ]</td>
<td>[ 100 ]</td>
<td>( f_{(4)} )</td>
<td>0.2857</td>
<td>0.2857 x ( C_{(Cal)} )</td>
</tr>
<tr>
<td>Std. (5)</td>
<td>[ 60 ]</td>
<td>[ 95 ]</td>
<td>( f_{(5)} )</td>
<td>0.3871</td>
<td>0.3871 x ( C_{(Cal)} )</td>
</tr>
<tr>
<td>Std. (6)</td>
<td>[ 55 ]</td>
<td>[ 50 ]</td>
<td>( f_{(6)} )</td>
<td>0.5238</td>
<td>0.5238 x ( C_{(Cal)} )</td>
</tr>
</tbody>
</table>

The concentration of Fibronectin in the standards \( C_{(i)} \) (g/L) is calculated from the Fibronectin concentration \( C_{(Cal)} \) (g/L) stated for the specific lot of the calibrator \( N \) Protein Standard PY and the relative concentration factor \( f_{(i)} \) by multiplication, i.e. \( C_{(i)} = f_{(i)} x C_{(Cal)} \). Please see the Analytical Value Sheet for the exact \( C_{(Cal)} \) value of the current lot.

**Protocol Information**

<table>
<thead>
<tr>
<th>Chem. Name</th>
<th>Fibronectin</th>
<th>Units</th>
<th>( g/L )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lot Number</td>
<td>XXXXXXX</td>
<td>Protocol</td>
<td>Non-Competitive Nephelometric</td>
</tr>
<tr>
<td>Reagent Serial Number</td>
<td>XXXXXXXXX</td>
<td>Reagent Expiration Date</td>
<td>XX/XX/XX</td>
</tr>
<tr>
<td>Sample or Dilution Volume</td>
<td>10 ( \mu L )</td>
<td>Gain</td>
<td>1</td>
</tr>
<tr>
<td>Reaction Buffer Volume</td>
<td>0 ( \mu L )</td>
<td>Cal. Dilution</td>
<td>1:1</td>
</tr>
<tr>
<td>Compartment A Volume</td>
<td>38 ( \mu L )</td>
<td>Sample Dilution</td>
<td>1:5</td>
</tr>
<tr>
<td>Compartment B Volume</td>
<td>195 ( \mu L )</td>
<td>Reaction Time</td>
<td>1.5 minutes</td>
</tr>
<tr>
<td>Calibrator Levels/Replicates</td>
<td>6/2</td>
<td>Calibrator Set Points</td>
<td>Lot-specific entries</td>
</tr>
</tbody>
</table>

XX Defined by the user.
User-Defined Model: Four Parameter Logistic

**Note:**

The maximum volume of compartment “A” and “B” of the UDR cartridge is 8.0 mL, and it has a dead volume of 250 \( \mu L \). While the system dispenses 195 \( \mu L \) of buffer from compartment B according to the protocol described above, it actually aspirates a total volume of 200 \( \mu L \) to avoid any possibilities of liquid contamination. Thus when the cartridge is maximally filled approximately 35 tests can be run. It is advisable to monitor the reagent usage, and refill compartment A and B when appropriate until the full 300 tests from the cartridge have been utilized.