Application Note
Guideline for Determination of
C-Reactive Protein (CRP) in Serum/Plasma
on Cobas MIRA Plus

General information

Intended use
The Application Note is intended for the quantitative determination turbidimetry of C-reactive protein in human sample material by turbidimetry on Cobas MIRA Plus (1, 2).

Measuring range
Approximately 5-160 mg/L depending on the specific lot of the calibrator.

Reference interval
0-5 mg/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.

<table>
<thead>
<tr>
<th>Code No.</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody</td>
<td>Q 0329 DakoCytomation Polyclonal Rabbit Anti-Human C-Reactive Protein</td>
</tr>
<tr>
<td>Reaction buffer</td>
<td>S 2011 DakoCytomation Reaction Buffer 4</td>
</tr>
<tr>
<td>Diluent</td>
<td>S 2005 DakoCytomation Dilution Buffer 1</td>
</tr>
<tr>
<td>Calibrator</td>
<td>X 0923 DakoCytomation Human Serum C-Reactive Protein Calibrator</td>
</tr>
<tr>
<td>Controls</td>
<td>X 0925 DakoCytomation Human Serum C-Reactive Protein Low Control</td>
</tr>
<tr>
<td></td>
<td>X 0926 DakoCytomation Human Serum C-Reactive Protein High Control</td>
</tr>
</tbody>
</table>

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

Calibrator
Dilution of standards is performed automatically by the instrument.

Reaction buffer
The reaction buffer is ready for use.

Antibody
The antibody solution is ready for use.
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.

Capacity: 1 mL of prediluted antibody is equivalent to approximately 40 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 instrument supplier or DakoCytomation Technical Service.
Performance Data

Sensitivity
An OD value of approximately 0.19 on Cobas MIRA Plus corresponds to a concentration around 160 mg/L C-reactive protein.

Detection limit
The detection limit is estimated to 2.7 mg/L.

Precision
The precision was estimated by testing samples at 3 different C-reactive protein (CRP) levels by ANOVA analysis of 6 runs each with a new calibration and with 6 determinations in each run.

<table>
<thead>
<tr>
<th>Sera</th>
<th>CRP 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></td>
</tr>
<tr>
<td>1</td>
<td>10.1</td>
<td>0.42</td>
<td>0.37</td>
<td>0.56</td>
</tr>
<tr>
<td>2</td>
<td>31.0</td>
<td>1.07</td>
<td>0.80</td>
<td>1.34</td>
</tr>
<tr>
<td>3</td>
<td>96.1</td>
<td>2.36</td>
<td>0.00</td>
<td>2.36</td>
</tr>
</tbody>
</table>

Accuracy
A recovery of C-reactive protein of 90-110% can be expected for DakoCytomation Human Serum C-Reactive Protein Low Control, code No. X 0925, and DakoCytomation Human Serum C-Reactive Protein High Control, code No. X 0926.

Linearity
The assay is linear in the range 5-160 mg/L.

Security range
No antigen excess is found for C-reactive protein concentrations up to 2000 mg/L (highest concentration tested).

Interference
No interference is found at concentrations up to 2.5 g/L of hemoglobin, 600 mg/L of bilirubin, and 7.5 g/L of triglyceride.
All drugs described in reference 5 were investigated according to the recommendations in reference 5. No interference was observed, except for Intralipid.

Method comparison
Determination of C-reactive protein according to this Application Note was compared with other commercial turbidimetric assays. Data are available on request.

References
**Instrument Settings (Software Version 9215)**

<table>
<thead>
<tr>
<th>P2</th>
<th>TESTS ROUTINE</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP</td>
<td>/15-JUL-95</td>
</tr>
</tbody>
</table>

**GENERAL**
- MEASUREMENT MODE: ABSORB
- REACTION MODE: D-R-S-SR1
- CALIBRATION MODE: EXPONENT5
- REAGENT BLANK: REAG/DIL
- CLEANER: NO
- WAVELENGTH: 340 nm
- DECIMAL POSITION: 2
- UNIT: mg/l

**ANALYSIS**
- NAME: DIL1
- FACTOR: 4.00
- TIME: NO
- STD: MAIN DIRECT
- MAIN STD: \((4.00 \times \text{C(cal)})^2\)/mg/l
- POS: 1
- FACT. STD-1: 1.28 2: 32.00 3: 16.00 4: 8.00 5: 5.33 6: 4.00 7: NO 8: NO
- POST DIL. FACTOR: NO
- CONC. FACTOR: NO
- SAMPLE CYCLE: 1
- VOLUME: 50.0 µl
- DILUENT NAME: H2O
- VOLUME: 5.0 µl
- REAGENT CYCLE: 1
- VOLUME: 150 µl
- START R1 CYCLE: 5
- VOLUME: 25.0 µl
- DILUTION NAME: H2O
- VOLUME: 5.0 µl

**CALCULATION**
- SAMPLE LIMIT: NO
- REAC. DIRECTION: INCREASE
- CHECK: ON
- ANTIGEN EXCESS: NO
- CONVERS. FACTOR: 1.00000
- OFFSET: 0.00000
- TEST RANGE LOW: OFF
- HIGH: ON
- NORM. RANGE LOW: NO
- HIGH: NO
- NUMBER OF STEPS: 1
- CALC. STEP A: ENDPOINT
- READINGS FIRST: 4 LAST: 16

**CALIBRATION**
- INTERVAL: ON REQUEST
- BLANK
- REAG. RANGE LOW: NO
- HIGH: NO
- BLANK RANGE LOW: NO
- HIGH: NO
- STANDARDS\(^*\)
  1: 5.09 2: 20.37 mg/l
  3: 40.75 4: 81.50 mg/l
  5: 122.33 6: 163.00 mg/l
- POS: 7: NO 8: NO
- REPLICATE: SINGLE
- DEVIATION: 5.0 %
- CORRECTION STD: NO
- CONTROL
  - CS1 POS: NO
  - CS2 POS: NO
  - CS3 POS: NO

DIL 1 – Code No. S 2005
REAGENT – Code No. S 2011
START R1 – Code No. Q 0329, undiluted.

\(^*\) Calibrator, code No. X 0923. The concentration is calculated as the factor times the calibrator value for the specific lot ($C_{\text{cal}}$, stated in mg/L on the X 0923 Analytical Value Sheet).

\(^*\) These values are not input. They are calculated automatically by the instrument from the "MAIN STD" and "FACT.STD" in the "ANALYSIS" section of the instrument settings. The values vary slightly with the calibrator value.