Procalcitonin Assay

REF: 567 001
R1 buffer 1 x 14 ml
R2 Latex 1 x 4.7 ml

Intended Use
Spectrum Procalcitonin Assay is for the quantitative determination of Procalcitonin Assay(PCT) in serum samples, EDTA or lithium heparin plasma samples by latex enhanced immunoturbidimetric method.

Background
Procalcitonin (PCT) is a 116 amino acid protein, the prohormone of calcitonin. Whereas hormonally active calcitonin is produced exclusively in the C-cells of the thyroid gland after specific intracellular proteolytic process of the prohormone PCT, PCT is ubiquitously and uniformly expressed in multiple tissues throughout the body in response to sepsis. In healthy conditions, the PCT levels in the circulation are very low (< 0.05 ng/ml). Elevated circulating levels of PCT are important indicators in response to microbial infections and a powerful tool in the early detection of sepsis.

Method
Turbidimetric method.

Test Principle
PCT Assay is based on a latex enhanced immunoturbidimetric assay.PCT proteins in the sample bind to the specific anti-PCT antibody, which is coated on latex particles, and causes agglutination. The degree of the turbidity caused by agglutination can be measured optically and is proportional to the amount of PCT in the sample. The instrument calculates the PCT concentration of a sample by interpolation of the obtained signal of a 6-point calibration curve.

Reagents

Reagent 1 (R1 Buffer)
Tris buffer 100 mmol/L

Reagent 2 (R2)
Suspension of anti-human PCT mouse monoclonal antibody coated latex particles (0.2%), ready to use

Calibrator: contain PCT in a human serum matrix lyophilized material.

Materials required but not provided with the kit
Any instrument with temperature control of 37 ± 0.5°C that is capable of reading absorbance accurately at 600 nm may be used. A saline solution is needed for calibration and high sample dilution. The PCT Calibrator Set and PCT Control Set are sold separately.

Precautions and Warnings
1. For “In Vitro Diagnostic Use”
2. Do not use the Reagent after the expiration date labeled on the outer box.
3. Assay calibration frequency is dependent on instrument used. Additionally, the assay should be recalibrated and controls run with each new lot of reagents.
4. Avoid ingestion and contact with skin and eyes.
5. Specimens containing human sourced materials should be handled as if potentially infectious using safe laboratory procedures, such as those outlined in Biosafety in Microbiological and Biomedical Laboratories.
6. The reagent contains < 0.1% sodium azide, NaN3, as preservative. Sodium azide may react with lead and copper plumbing to form highly explosive metal azide. On disposal, flush with a large volume of water to prevent azide buildup.

Reagent Preparation, Storage and Stability
The reagents are ready-to-use and stable when stored at 2 – 8 °C. until the expiry date labeled on the bottles.

DO NOT FREEZE

Specimen Collection and Preservation
The PCT Assay is formulated for use with serum or lithium heparin and EDTA plasma. For monitoring purpose, the same sample matrix should always be used. PCT values measured in arterial blood are about 4% higher than from venous blood. PCT increases about 3 hours after bacterial infection, reaching maximum values after 6-12 hours with a half life of 25 to 30 hours. PCT is relative stable in both plasma and serum samples, and no special requirements for pre-analytical sample handling.

Samples stored at 4°C retain >90 percent for several days, whereas samples stored at room temperature for 24 hours retain 80 percent of their initial concentration. Stored at -20°C, it is stable for 6 months. 3 cycles of Freeze/thaw has <2% loss of PCT in sample.

Stability: several days at 4°C
24 hours at room temp.
6 months at -20°C

System Parameters

Wavelength: 600 nm
Optical path: 1 cm
Assay type: Turbidimetric
Sample: Reagent Ratio 1:60
Temperature: 37 °C and 20 – 25 °C
Zero adjustment: Reagent or Sample blank
Linearity: 80 ng/mL

Procedure

<table>
<thead>
<tr>
<th>Calibrator</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1(buffer)</td>
<td>270 μl</td>
</tr>
<tr>
<td>Sample</td>
<td>-----</td>
</tr>
<tr>
<td>Calibrator</td>
<td>25 μl</td>
</tr>
</tbody>
</table>

Mix and incubate for 4.5 minutes at 37 °C, then add

| R2 Latex | 90 μl |

Mix and incubate for 2 minutes, measure absorbance of specimen A1, then after 3 minutes record absorbance of specimen A2

Calculation

Generate a reference curve by successive 1 : 2 dilutions of standard in saline (At Least 4 points are recommended). Use Saline as zero point. Determine Δ absorbance of the sample and each standard as following:

Δ absorbance of sample = (A2 - A1) sample
Δ absorbance of each standard = (A2 - A1) for each standard
Plot the calibration curve and obtain the result.
Calibration

The Spectrum PCT Assay should be calibrated using Spectrum PCT Calibrator. PCT concentration in sample is determined from a calibration curve obtained from the PCT Calibrators. The PCT Calibrators are lyophilized powder and require reconstitution with DI water before use. Biweekly calibration is recommended.

Quality Control

Good laboratory practice recommends the use of control materials. Users should follow the appropriate federal, state and local guideline concerning the running of external quality control. To ensure adequate quality control, normal and abnormal controls with known values should be run as unknown samples. PCT Control are lyophilized powder and require reconstitution with cold DI water before use.

Linearity

The linearity of the assay is from 0.17-60 ng/mL. Results that exceed 60 ng/mL should be diluted with saline and retested.

Interfering Substances

The substances normally present in the plasma were tested. Less than 10% deviation was produced when tested up to the concentrations shown below:

<table>
<thead>
<tr>
<th>Substance</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascorbic Acid</td>
<td>10 mM</td>
</tr>
<tr>
<td>Bilirubin, free</td>
<td>40 mg/dL</td>
</tr>
<tr>
<td>Bilirubin, conjugated</td>
<td>40 mg/dL</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>400 mg/dL</td>
</tr>
<tr>
<td>Triglyceride Factor</td>
<td>1000 mg/dL</td>
</tr>
</tbody>
</table>

Analytical characteristics

Precision

Within-run precision of the Diazyme PCT Assay was evaluated. In the study, three levels of PCT controls containing 0.5 ng/mL, 2.14 ng/mL and 13.13 ng/mL PCT respectively were tested with 20 replicates in one run.

<table>
<thead>
<tr>
<th>Level</th>
<th>Number of Data Points</th>
<th>Mean (ng/mL)</th>
<th>SD (ng/mL)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1</td>
<td>20</td>
<td>0.50</td>
<td>0.036</td>
<td>7.2%</td>
</tr>
<tr>
<td>Level 2</td>
<td>20</td>
<td>2.14</td>
<td>0.069</td>
<td>3.2%</td>
</tr>
<tr>
<td>Level 3</td>
<td>20</td>
<td>13.13</td>
<td>0.353</td>
<td>2.7%</td>
</tr>
</tbody>
</table>

Accuracy

The performance of this assay was compared with the performance of a legally marketed PCT Assay using lithium heparin plasma samples. For the 41 plasma samples with PCT ranging from 0.18 ng/mL to 58.44 ng/mL, the correlation coefficient between the two methods was 0.9141, slope was 0.9815, and y intercept was 0.3137.

Limit of Quantitation

The Limit of Quantitation (LOQ) of the PCT Assay was determined to be 0.17 ng/mL.

Expected Values

1. Spectrum Procalcitonin < 0.5 ng/mL: systemic infection (sepsis) is not likely.
2. Local bacterial infection is possible; PCT > 0.5 ng/mL and < 2 ng/mL: systemic infection (sepsis) is possible but other conditions are known to increase PCT as well; PCT > 2 ng/mL and < 10 ng/mL: systemic infection (sepsis) is likely, unless other cause are known; PCT > 10 ng/mL: important systemic inflammatory response, almost exclusively due to severe bacterial or septic shock.

Limitation

Samples with PCT level exceeding the linearity limit of 60 ng/mL should be diluted with negative sample. Rerun the tests and multiply the results by the dilution factors. As with any latex turbidimetric immunoassays, Diazyme PCT Assay runs should be followed with appropriate and thorough wash steps. Please consult specific instrument manuals for further information.

References


ORDERING INFORMATION

<table>
<thead>
<tr>
<th>CATALOG NO.</th>
<th>QUANTITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>567 001</td>
<td>50 test</td>
</tr>
</tbody>
</table>