Liquid Reagents – ready to use

CALCUM
CPC with ATCS*
2 Reagents

Diagnostic reagent for quantitative in vitro determination of calcium in human serum, plasma or urine on photometric systems

REF Cont.

D99097 5 x 100 mL 4 x 100 mL Reagent 1
1 x 100 mL Reagent 2

D95098 5 x 50 mL 4 x 50 mL Reagent 1
1 x 50 mL Reagent 2

Additionally offered:

D95094 1 x 3 mL Calcium Standard
D98485 5 x 3 mL Calibrator
D98481 12 x 5 mL Control normal
D98482 12 x 5 mL Control abnormal
D08581 12 x 5 mL Urine Ctrl. norm.
D08582 12 x 5 mL Urine Ctrl. abnorm.

TEST PARAMETERS

Method: Colorimetric, Endpoint, Increasing Reaction, CPC
Wavelength: 570 nm (550 - 590 nm), Hg 578 nm
Temperature: 20 – 25 °C, 37 °C
Sample: Serum or heparinized plasma, acidified urine (do not use EDTA plasma)
Linearity: up to 25 mg/dL (6.25 mmol/L) on Hitachi 911
Sensitivity: The lower limit of detection is 0.2 mg/dL (0.05 mmol/L)

* Advanced Turbidity Clearing System; minimizes turbidity caused by lipemia

REAGENT COMPOSITION

<table>
<thead>
<tr>
<th>COMPONENTS</th>
<th>CONCENTRATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent 1</td>
<td>pH 10.7</td>
</tr>
<tr>
<td>Ethanolamine</td>
<td>750 mmol/L</td>
</tr>
<tr>
<td>Detergents</td>
<td></td>
</tr>
<tr>
<td>Reagent 2</td>
<td>pH 1.1</td>
</tr>
<tr>
<td>o-Cresolphthalein complexone</td>
<td>0.13 mmol/L</td>
</tr>
<tr>
<td>8-Hydroxychinoline</td>
<td>35 mmol/L</td>
</tr>
<tr>
<td>Hydrochloric acid</td>
<td>100 mmol/L</td>
</tr>
</tbody>
</table>

REAGENT PREPARATION

Substrate Start: Reagents are ready for use.

Sample Start:
Mix 4 parts of Reagent 1 with 1 part of Reagent 2. (= Working Reagent)

REAGENT STABILITY AND STORAGE

Conditions: protect from light
close immediately after use, otherwise the pH decreases because of CO₂ absorption from the air.
do not freeze the reagents!

Substrate Start:
Storage: at 2 – 8°C
Stability: up to the expiration date

Sample Start (Working Reagent):
Stability in closed vials: at 2 – 8 °C 3 days
                       at 16 – 25°C 3 days

SAMPLE PREPARATION

Urine: add 10 mL of conc. HCl to 24 h Urine and heat the specimen to dissolve calcium oxalate

SAMPLE STABILITY AND STORAGE [5]

In serum/plasma: at 20 – 25 °C 7 days
                at 2 – 8 °C 3 weeks
                at -20 °C 8 months
In urine: at 20 – 25 °C 2 days
         at 2 – 8 °C 4 days
         at -20 °C 3 weeks

Mix and read absorbance A1 after reagent blank after 5 - 30 min. at 20-25°C/37°C.
Then add:
Reagent 2 250 µL 250 µL 250 µL
Mix and read absorbance A2 against reagent blank after 5 - 30 min. at 20-25°C/37°C.

ΔA = (A2-A1) sample or Std./Cal.

MANUAL TEST PROCEDURE

Substrate Start:

Pipette into test tubes Blank Std./Cal. Sample
Sample - - 20 µL
Std./Cal. - 20 µL -
Dist. water 20 µL - -
Reagent 1 1000 µL 1000 µL 1000 µL
Mix and read absorbance A1 against reagent blank after 5 - 30 min. at 20-25°C/37°C.
Then add:
Reagent 2 250 µL 250 µL 250 µL
Mix and read absorbance A2 against reagent blank after 5 - 30 min. at 20-25°C/37°C.

Sample Start:

Pipette into test tubes Blank Std./Cal. Sample
Sample - - 20 µL
Std./Cal. - 20 µL -
Dist. water 20 µL - -
Working Reagent 1000 µL 1000 µL 1000 µL
Mix and read absorbance against reagent blank after 5 - 30 min at 20-25°C/37°C.

STANDARD
(has to be ordered separately)
Concentration: 10 mg/dL (2.5 mmol/L)
Storage: 2 – 25°C
Stability: up to the expiration date
CLOSE IMMEDIATELY AFTER USE!

INTERFERING SUBSTANCES
no interference up to:
ascorbic acid 30 mg/dL
bilirubin 40 mg/dL
hemoglobin 500 mg/dL
triglycerides 2000 mg/dL
magnesium 15 mg/dL
Strontium salts in medicine may lead to strongly increased calcium values.
CALCULATION (light path 1 cm)

\[
\text{Calcium} \ [\text{mg/dL}] = \frac{\Delta A_{\text{Sample}}}{\Delta A_{\text{Std./Cal.}}} \times \text{Conc. Std./Cal.}[\text{mg/dL}]
\]

UNIT CONVERSION

\[
\text{mg/dL} \times 0.2495 = \text{mmol/L}
\]

REFERENCE RANGE \(^{[2]}\)

<table>
<thead>
<tr>
<th></th>
<th>mg/dL</th>
<th>mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>serum/plasma:</td>
<td>8.6 - 10.3</td>
<td>2.15 - 2.57</td>
</tr>
<tr>
<td>urine:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women:</td>
<td>&lt; 250</td>
<td>&lt; 6.24</td>
</tr>
<tr>
<td>Men:</td>
<td>&lt; 300</td>
<td>&lt; 7.49</td>
</tr>
</tbody>
</table>

* Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

TEST PRINCIPLE

Cresolphthalein complexone (CPC) reacts with Calcium ions in alkaline solution forming a violet colour. The intensity of the violet color is proportional to the calcium concentration in the sample. Interference by magnesium is eliminated by addition of 8-hydroxyquinoline.

PERFORMANCE CHARACTERISTICS

LINEARITY

The assay is linear between 0.2 - 25 mg/dL (0.05 - 6.25 mmol/L) on Hitachi 911. Above this concentration, samples should be diluted 1 + 1 with NaCl solution (9 g/L) and reassayed multiplying the result by 2.

PRECISION (at 37°C)

<table>
<thead>
<tr>
<th></th>
<th>Mean [mg/dL]</th>
<th>SD [mg/dL]</th>
<th>CV [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intra-assay n = 20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample 1</td>
<td>6.18</td>
<td>0.06</td>
<td>0.84</td>
</tr>
<tr>
<td>Sample 2</td>
<td>9.94</td>
<td>0.10</td>
<td>1.02</td>
</tr>
<tr>
<td>Sample 3</td>
<td>13.5</td>
<td>0.11</td>
<td>0.81</td>
</tr>
</tbody>
</table>

Inter-assay Mean [mg/dL] SD [mg/dL] CV [%] Sample 1 6.31 0.09 1.38 Sample 2 10.1 0.10 1.04 Sample 3 13.4 0.08 0.63

METHOD COMPARISON

A comparison between Dialab Calcium (y) and a commercially available test (x) using 82 samples gave following results:

\[
y = 0.98 x + 0.11; r = 0.999.
\]

QUALITY CONTROL

All controls with Calcium values determined by this method can be used.

We recommend:

<table>
<thead>
<tr>
<th>REF</th>
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</tr>
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<tbody>
<tr>
<td>D98481</td>
<td>12 x 5 mL</td>
</tr>
<tr>
<td>D98482</td>
<td>12 x 5 mL</td>
</tr>
<tr>
<td>D08581</td>
<td>12 x 5 mL</td>
</tr>
<tr>
<td>D08582</td>
<td>12 x 5 mL</td>
</tr>
</tbody>
</table>

CALIBRATION

The assay requires the use of a Calcium Standard or a Calcium Calibrator. We recommend:

<table>
<thead>
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</tr>
</thead>
<tbody>
<tr>
<td>D95094</td>
<td>1 x 3 ml</td>
</tr>
<tr>
<td>D98485</td>
<td>5 x 3 ml</td>
</tr>
</tbody>
</table>

AUTOMATION

Special adaptations for automated analyzers can be made on request.

WARNINGS AND PRECAUTIONS

1. Reagent 1 is irritating: Xi R36: Irritating to eyes. S2: keep out of the reach of children. S25: Avoid contact with eyes. S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

2. Reagent 2: S24/25: Avoid contact with skin and eyes.

3. As calcium is an ubiquitous ion, essential precaution must be taken against accidental contamination. Only use disposable materials.

4. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents.

WASTE MANAGEMENT

Please refer to local legal requirements.

REFERENCES


