Glucose Hexokinase FS*

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

Order Information

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Kit size</th>
<th>R1</th>
<th>20 mL</th>
<th>20 mL</th>
<th>1</th>
<th>3 mL</th>
<th>Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 2511 99 10 021</td>
<td></td>
<td>4</td>
<td>+</td>
<td>1</td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 2511 99 10 026</td>
<td></td>
<td>5</td>
<td>80 mL</td>
<td>+</td>
<td>1</td>
<td>1</td>
<td>100 mL</td>
</tr>
<tr>
<td>1 2511 99 10 023</td>
<td></td>
<td>1</td>
<td>800 mL</td>
<td>+</td>
<td>1</td>
<td>1</td>
<td>200 mL</td>
</tr>
<tr>
<td>1 2511 99 10 704</td>
<td></td>
<td>8</td>
<td>50 mL</td>
<td>+</td>
<td>8</td>
<td>2</td>
<td>12.5 mL</td>
</tr>
<tr>
<td>1 2511 99 10 917</td>
<td></td>
<td>8</td>
<td>60 mL</td>
<td>+</td>
<td>2</td>
<td>8</td>
<td>15 mL</td>
</tr>
<tr>
<td>1 2500 99 10 030</td>
<td></td>
<td>6</td>
<td>+</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Summary [1,2]
Measurement of glucose concentration in serum or plasma is mainly used in diagnosis and monitoring of treatment in diabetes mellitus. Other applications are the detection of neonatal hypoglycemia, the exclusion of pancreatic islet cell carcinoma as well as the evaluation of carbohydrate metabolism in various diseases.

Method
Enzymatic UV test using hexokinase

Principle
Glucose + ATP $\rightarrow$ Glucose-6-phosphate + ADP

Glucose-6-phosphate $\rightarrow$ NAD$^+$ - G6P-DH $\rightarrow$ Gluconate-6-P + NADH + H$^+$

Reagents

Components and Concentrations

R1: TRIS buffer pH 7.8 100 mmol/L
Mg$^{2+}$ 4 mmol/L
ATP 2.1 mmol/L
NAD 2.1 mmol/L
R2: Mg$^{2+}$ 4 mmol/L
Hexokinase (HK) $\geq$ 7.5 kU/L
Glucose-6-phosphatedehydrogenase $\geq$ 7.5 kU/L
(G6P-DH)

Standard: 100 mg/dL (5.55 mmol/L)

Storage Instructions and Reagent Stability
Reagents and standard are stable up to the end of the indicated month of expiry, if stored at 2 – 8°C, protected from light and contamination is avoided. Do not freeze the reagents!

Warnings and Precautions
1. The reagents contain sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
2. Reagent 2 contains animal material. Handle the product as potentially infectious according to universal precautions and good laboratory practice.
3. In very rare cases, samples of patients with gammapathy might give false results [6].
4. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents. For diagnostic purposes, the results should always be assessed with the patient’s medical history, clinical examinations and other findings.
5. For professional use only!

Waste Management
Please refer to local legal requirements.

Materials required but not provided
NaCl solution 9 g/L
General laboratory equipment

Reagent Preparation
The standard is ready to use.

Substrate Start
The reagents are ready to use.

Sample Start
Mix 4 parts of R1 with 1 part of R2
(eg. 20 mL R1 + 5 mL R2) = mono-reagent
Stability: 3 months at 2 – 8°C
3 weeks at 15 – 25°C
The mono-reagent must be protected from light.

Specimen
Serum, plasma or urine
Separate from cellular content at the latest 1h after blood collection.
Stability in plasma after addition of a glycolytic inhibitor (fluoride, monoiiodacetate, mannose) [3]:
2 days at 20 – 25°C
7 days at 4 – 8°C
1 day at -20°C
Stability in serum (separated from cellular contents, hemolysis free) without adding a glycolytic inhibitor [2,4]:
8 h at 25°C
24 h at 4°C
Stability in urine [3]:
2 h at 20 – 25°C
24 h at 4 – 8°C
Only freeze once!
Discard contaminated specimens!

Assay Procedure
Application sheets for automated systems are available on request.

Wavelength 340 nm, Hg 334 nm, Hg 365 nm
Optical path 1 cm
Temperature 20 – 25 °C/37 °C
Measurement Against reagent blank

Substrate Start

Sample or standard Blank Sample or standard
Dist. water 10 µL 10 µL
Reagent 1 1000 µL 1000 µL
Mix and incubate 1–5 min. at 20 – 25 °C/37 °C. Read absorbance A1, then add:
Reagent 2 250 µL 250 µL
Mix, incubate 5 min. at 37 °C or 10 min. at 20 – 25 °C. Read absorbance A2 against reagent blank within 30 min.

$\Delta A = (A2 – A1)$ Sample/standard

Sample Start

Sample or standard Blank Sample or standard
Dist. water 10 µL 10 µL
Mono-reagent 1000 µL 1000 µL
Mix, incubate 5 min. at 37 °C or 10 min. at 20 – 25 °C. Read absorbance against reagent blank within 30 min.

$\Delta A = A$ Sample/Standard
Note:
The pipetting scheme with sample start is recommended only for analyzers with correction of sample blank (e.g. by bichromatic measurement). Samples often show relatively high absorbances at the measurement wavelengths which tend to show falsely high glucose values when working with sample start. The given calculation factors cannot be used for bichromatic measurements.

Calculation

With factor

Multiply $\Delta A$ by the corresponding factor $f$ from the table below in order to calculate the glucose concentration.

<table>
<thead>
<tr>
<th>Substrate start</th>
<th>$f$ [mg/dL]</th>
<th>$f$ [mmol/L]</th>
</tr>
</thead>
<tbody>
<tr>
<td>340 nm</td>
<td>361</td>
<td>20.0</td>
</tr>
<tr>
<td>Hg 334 nm</td>
<td>367</td>
<td>20.5</td>
</tr>
<tr>
<td>Hg 365 nm</td>
<td>667</td>
<td>37.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample start</th>
<th>$f$ [mg/dL]</th>
<th>$f$ [mmol/L]</th>
</tr>
</thead>
<tbody>
<tr>
<td>340 nm</td>
<td>289</td>
<td>16.0</td>
</tr>
<tr>
<td>Hg 334 nm</td>
<td>294</td>
<td>16.4</td>
</tr>
<tr>
<td>Hg 365 nm</td>
<td>535</td>
<td>29.7</td>
</tr>
</tbody>
</table>

With standard or calibrator

Glucose $\times 0.05551 = \text{Glucose [mmol/L]}$

Conversion factor

Glucose [mg/dL] x 0.05551 = Glucose [mmol/L]

Calibrators and Controls

For calibration of automated photometric systems, DiaSys TruCal U calibrator is recommended. The calibration values of this calibrator have been made traceable to the reference method gas chromatography – isotope dilution mass spectrometry (GC-IDMS). For internal quality control DiaSys TruLab N, P and TruLab Urine controls should be assayed. Each laboratory should establish corrective action in case of deviations in control recovery.

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Kit size</th>
</tr>
</thead>
<tbody>
<tr>
<td>TruCal U 5 9100 99 10 063</td>
<td>20 x 3 mL</td>
</tr>
<tr>
<td>TruLab N 5 9000 99 10 062</td>
<td>20 x 5 mL</td>
</tr>
<tr>
<td>TruLab P 5 9050 99 10 061</td>
<td>20 x 5 mL</td>
</tr>
<tr>
<td>TruLab Urine Level 1 2 5 9170 99 10 061</td>
<td>20 x 5 mL</td>
</tr>
<tr>
<td>TruLab Urine Level 2 5 9180 99 10 061</td>
<td>20 x 5 mL</td>
</tr>
</tbody>
</table>

Performance Characteristics

Measuring range

The test has been developed to determine glucose concentrations within a measuring range from 2 – 900 mg/dL (0.1 – 50 mmol/L) measured at 365 nm, respectively within a measuring range from 2 – 500 mg/dL (0.1 – 28 mmol/L) measured at 334/340 nm. When values exceed these ranges serum and plasma samples should be diluted 1+2 with NaCl solution (9 g/L) and the result multiplied by 3, urine samples should be diluted 1+10 with dist. water and the results multiplied by 11.

Specificity/Interferences

No interference was observed by ascorbic acid up to 30 mg/dL, bilirubin up to 40 mg/dL, hemoglobin up to 500 mg/dL and lipemia up to 2000 mg/dL triglycerides, when worked with substrate start. For further information on interfering substances refer to Young DS [5].

Sensitivity/Limit of Detection

The lower limit of detection is 2 mg/dL (0.1 mmol/L).

Precision (at 37 °C)

<table>
<thead>
<tr>
<th>n</th>
<th>Mean [mg/dL]</th>
<th>SD [mg/dL]</th>
<th>CV [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>Sample 1</td>
<td>65.7</td>
<td>1.39</td>
</tr>
<tr>
<td>Sample 2</td>
<td>121</td>
<td>2.54</td>
<td>2.11</td>
</tr>
<tr>
<td>Sample 3</td>
<td>298</td>
<td>6.57</td>
<td>2.21</td>
</tr>
</tbody>
</table>

Method Comparison

A comparison of DiaSys Glucose Hexokinase FS (y) with a commercially available test (x) using 73 samples gave following results:

$y = 1.00 x + 0.00$ mg/dL; $r = 0.998$

Reference Range [1]

<table>
<thead>
<tr>
<th>[mg/dL]</th>
<th>[mmol/L]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cord blood</td>
<td>63 – 159</td>
</tr>
<tr>
<td>1 h</td>
<td>36 – 99</td>
</tr>
<tr>
<td>2 h</td>
<td>36 – 89</td>
</tr>
<tr>
<td>5 – 14 h</td>
<td>34 – 77</td>
</tr>
<tr>
<td>10 – 28 h</td>
<td>46 – 81</td>
</tr>
<tr>
<td>44 – 52 h</td>
<td>48 – 79</td>
</tr>
</tbody>
</table>

Children (fasting):

1 – 6 years | 74 – 127 | 4.1 – 7.0 |
7 – 19 years | 70 – 106 | 3.9 – 5.9 |

Adults (fasting):

Serum/plasma | 70 – 115 | 3.9 – 6.4 |

Urine:

< 15 mg/dL (0.84 mmol/L) (Value is based on an average quantity of urine of 1350 mL/day)

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

Literature