D-GLUCOSE + D-FRUCTOSE

INTENDED USE
Reagent for photometric determination of D-Glucose and D-Fructose (sum) in homogenous liquid samples using automated Thermo Scientific Arena or Gallery analyzer.

METHOD
Enzymatic test with Hexokinase (HK) and Phosphoglucone Isomerase (PGI) and Glucose-6-phosphate Dehydrogenase (G6P-DH).

PRINCIPLE OF THE PROCEDURE
D-Fructose + ATP ---HK---> Fructose-6-phosphate + ADP
D-Glucose + ATP ---HK---> Glucose-6-phosphate + ADP
Fructose-6-phosphate ---PGI---> Glucose-6-Phosphate
Glucose-6-phosphate + NAD" ---G6P-DH---> Gluconate-6-P + NADH + H+

REAGENT INFORMATION
Reagent 1 (R1) 3 x 16 ml
Reagent 2 (R2) 3 x 4.5 ml
Reagent 3 (R3) 3 x 4.5 ml

Note: Labels of reagents vials have two barcodes. For Arena analyzers, turn the short barcode on the left side to the barcode reader. For Gallery analyzers, turn the long barcode on the right side to the reading position of the reagent rack.

Sample preparation
If the sample has substances interfering the measurement, please handle it according to the following suitable preparation procedure:

- Use clear, colorless and practically neutral liquid samples directly.
- Filter or centrifuge turbid solutions.
- Degas samples containing carbon dioxide.
- Crush or homogenize solid or semi-solid samples.
- Weigh sufficient quantity of sample in a volumetric flask (take care of the measuring range), extract with water and filtrate, centrifuge or use Carrez clarification if necessary.
- Weigh sufficient quantity of fat containing samples into a volumetric flask (take care of the measuring range), extract with hot water. Cool to allow the fat to separate, make up the mark, place the volumetric flask in an ice bath for 15 min. and filter. Alternatively use Carrez clarification after extraction.
- Adjust acid samples to pH 8 by adding sodium or potassium hydroxide solution and incubate for approx. 15 min.
- Treat strongly colored samples with polyvinylpolypyrrolidone (PVPP e.g. 1 g/100 ml Sample).
- Carrez clarification:
  - Weigh sufficient quantity of the sample into a 100 ml volumetric flask which contains approx. 60 ml dist. water. Subsequently carefully add 5 ml Carrez-I-solution (potassium ferrocyanide, 85 mmol = 3.60 g K4[Fe(CN)6] × 3 H2O/100 ml), 5 ml Carrez-II-solution (zinc sulphate, 250 mmol = 7.20 g ZnSO4 × 7 H2O/100 ml) and 5 ml Carrez-III-solution (potassium hydroxide, 5 ml). Mix after each addition. Fill the volumetric flask with water to the mark, mix and filter.

TEST PROCEDURE
See a separate application for the Arena or Gallery analyzer.

Materials required but not provided
Distilled water (aseptic and free of heavy metals) and general laboratory equipment.

Sugar combination standard Cat no. 984380 (one level, water based) is not included in the kit.

Calibration
Water based Sugar combination standard can be used or other. Ordering code for Sugar combination standard is 984380 (3 x 3 ml). The standard is ready-to-use.

Calculate the sum of the D-Glucose and D-Fructose concentrations from the Sugar combination standard. Concentrations are stated in the package label of the standard.

Quality Control
Use quality control samples at least once a day and after each calibration and every time a new bottle of reagent is used. It is recommended to use two levels of controls. The control intervals and limits must be adapted to the individual laboratory requirements. The results of the quality control sample(s) should fall within the limits set by the laboratory.

Available controls:
Sugar combination standard can be used. If Sugar combination standard is used also for calibration, an additional internal control is recommended to be used.
CALCULATION OF RESULTS
The results are calculated automatically by the analyzer using a calibration curve.

Calibration Curve (example)

<table>
<thead>
<tr>
<th></th>
<th>Response (A)</th>
<th>Calc. conc. (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>0.009</td>
<td>0.000</td>
</tr>
<tr>
<td>Sugar std</td>
<td>1.133</td>
<td>49.85</td>
</tr>
</tbody>
</table>

Calibration factor of this example is 0.887.
Note that the calibration curve is lot dependent.

LIMITATIONS OF THE PROCEDURE

Interference
The determination is specific for D-Glucose and D-Fructose.

MEASURING RANGE
The test has been developed to determine D-Glucose and D-Fructose concentrations within a measuring range from 0.04 to 200 g/l.

PERFORMANCE CHARACTERISTICS
The results obtained in individual laboratories may differ from the performance data given.

<table>
<thead>
<tr>
<th></th>
<th>Mean 3.29 g/l</th>
<th>Mean 27.61 g/l</th>
<th>Mean 55.74 g/l</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SD</td>
<td>CV %</td>
<td>SD</td>
</tr>
<tr>
<td>Within run</td>
<td>0.082</td>
<td>2.5</td>
<td>0.229</td>
</tr>
<tr>
<td>Between run</td>
<td>-</td>
<td>-</td>
<td>0.431</td>
</tr>
<tr>
<td>Total</td>
<td>0.129</td>
<td>3.9</td>
<td>0.554</td>
</tr>
</tbody>
</table>

A precision study was performed using Arena 20XT for 5 days, with the number of measurements being n = 50.

WASTE MANAGEMENT
Please refer to local legal requirements. Empty the cuvette waste bin daily immediately after the analysis.

OTHER REMARKS
All results must be verified by laboratory quality control samples.
Manufacturer does not warrant that the product is error-free or will accomplish any particular result. In no event shall the manufacturer be liable for special, incidental, indirect, punitive or consequential damages (including, but not limited to, loss of profits, loss of goodwill, loss of data or loss of use damages) arising out of the use or disposition of the products.

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2010-06-04.

Changes from previous version
Gallery information added.
Samples and Performance characteristics updated.