D-Fructose/D-Glucose, UV method

Catalogue number: AK00041, 110 tests (manual) / 1100 tests (microplate)

Alternative Procedures

Total reducing sugars

This kit allows the quantification of D-fructose and D-glucose individually. However, in many cases it is unnecessary to differentiate between these sugars, allowing them to be quantified together (total reducing sugars) using a more rapid assay format, as follows:

Supplementary preparation step:

Gently shake Suspensions 3 and 4 to remove any enzyme that may have settled on the caps. Using a pipette, transfer the entire contents of Suspension 4 (PGI) into Suspension 3 (HK/G6P-DH). Mix the enzymes by gentle swirling. This HK/ G6P-DH/PGI mixture is now ready for use.

After performing this step, D-glucose and D-fructose cannot be measured individually with this kit reagent mixture.

Procedure

Wavelength: 340 nm

Cuvette: 1 cm light path (glass or plastic)

Temperature: ~ 25 °C

Final volume: 2.34 mL (D-glucose plus D-fructose) Sample solution: 4-80 µg of D-glucose plus D-fructose per cuvette (in 0.10-2.00 mL sample volume)

Read against air (without a cuvette in the light path) or against water

Pipette into cuvettes (mL)	**Blank**	**Sample**
Distilled water (at ~25 °C)	2.10	2.00
Sample	-	0.10
Solution 1 (imidazole buffer)	0.10	0.10
Solution 2 ($NADP^+$+ATP)	0.10	0.10
Mix, measure the absorbance of the above solutions (A1) after approx. 3 min and start the reaction by addition of		
Suspension 3+4 (HK+G6PDH + PGI))	0.04	0.04
Mix*, measure the absorbance of the solutions (A_{total}) at the end of the reaction (approx. 10 min). If the reaction has not stopped after 10 min, continue to read the absorbance at 2 min intervals until the absorbance remain the same over 2 min**		

Calculation

Determine the absorbances difference for both blank and sample (A_{total}-A_1). Subtract the absorbances difference of the blank from the absorbance difference of the sample, thereby obtaining $\Delta A_{D\text{-}fructose\ +D\text{-}glucose}$. The concentration of Total reducing sugars (D-fructose+D-glucose; g/L), based on the Abs of NADH at 340 nm (6300 L×mol^{-1}×cm^{-1}), are calculated as follows:

$$C= 0.6692 \times \Delta A_{D\text{-}fructose\ +D\text{-}Glucose} \quad [g/L]$$

Micro-volumes formats

This kit has been developed to work in cuvettes with a standard pathlength of 1 cm, as described in the respective “Product Brochure”. However, it can be adapted for use in 96-well microplates or in auto-analysers (micro-volume formats) with minimal assay optimisation. Basically, the assay volumes for the cuvette format have to be reduced approximately 10-fold for use in microplate format or in auto-analyser format. However, when using these micro-volume formats, you must be aware that the radiation pathlength is usually smaller than the standard cuvette pathlength of 1 cm. Thus, to perform the calculation of the amount of analyte in the samples under analysis follow one of the three strategies described in the section below.

Auto-analyser procedure (for reducing sugars)

This kit is appropriate for the preparation of 254.1 mL of reagent (equivalent to 1155 reactions of 0.230 mL). Reagent preparation is accomplished as follows:

Preparation of R1:

Component	**Volume**
Solution 1	1.0 mL
Solution 2 (after addition of 12 mL of H2O)	1.0 mL
Suspension 4 (swirl before use)	0.2 mL
PVP solution (10 mg/mL) or Distilled water	1.0 mL
Distilled water	18.0 mL

Preparation of R2:

Component	Volume
Suspension 3 (swirl before use)	0.2 mL
Distilled water	1.9 mL
Total	2.1 mL

Example Procedure:

	Volume
R1	0.200 mL
Sample	0.002 mL*
R2	0.020 mL

Reaction time: 10 min at 25 °C or 5 min at 37 °C

Wavelength: 340 nm

Prepared reagent stability: > 7 days when refrigerated

Calculation: endpoint

Reaction direction: increase

Linearity: up to 108 µg/mL of D-glucose + D-fructose in final reaction mixture

∗ If AU values are higher than 2, please dilute the sample with distilled water accordingly.

Strategies for analyte calculation

Auto-analysers use reaction volumes of approximately 0.315 mL and pathlengths from 4 to 8 mm, which is similar to a standard 96-well microplate in which the same reaction volume would have a pathlength of 6 or 7 mm (similar assay volumes). Therefore, in both formats (96-well microplate and auto-analysers systems), the calculation of the analyte must be done by one of the three possible methods described below:

1. Using the pathlength conversion factor

This is the easiest method to perform the calculation of the analyte. However, it requires a microplate reader with pathlength conversion capacity, i.e., the apparatus can detect the pathlength of each well and convert the individual readings to a 1 cm pathlength (cuvette format). In the case of auto-analysers, the absorbance readings should be directly converted to a 1 cm pathlength. This will allow the calculation of the analyte content as described in the “Product Brochure”, provided with the kit and available at the NZYTech website.

2. Using one standard curve

In this method, it is necessary to perform a standard curve of the analyte on each microplate that contains the test samples, or in the auto-analyser, and calculate the result from the standard curve of analyte concentration vs. absorbance. The standard curve can be performed by using the control solution provided in the kit.

3. Using two standard curves

The most complicated method is to perform standard curves of the analyte in both the cuvette format (i.e. with a 1 cm of radiation pathlength) and the 96-well microplate or auto-analyser formats, and use these results to obtain a mean conversion factor between the cuvette procedure values and the alternative procedure values. The standard curves can be performed by using the control solution provided in the kit.

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