# D-Fructose/D-Glucose, UV method

## 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 $L \times mol^{-1} \times cm^{-1}$), 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 |
| Total | 21.2 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** | 0.010 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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