



D-Fructose/D-Glucose (K-FRUGL) Procedure for ChemWell®-T Auto-Analyser

Requirements:

- D-Fructose/D-Glucose Assay Kit (K-FRUGL) (provides ~ 1100 assays).
- K-FRUGL (GLUCOSE) and K-FRUGL (TOTAL) ChemWell®-T assay files and the K-FRUGL (FRUCTOSE) ChemWell®-T indices file.
- Use in association with the D-Fructose/D-Glucose Assay Kit (K-FRUGL) product data booklet.

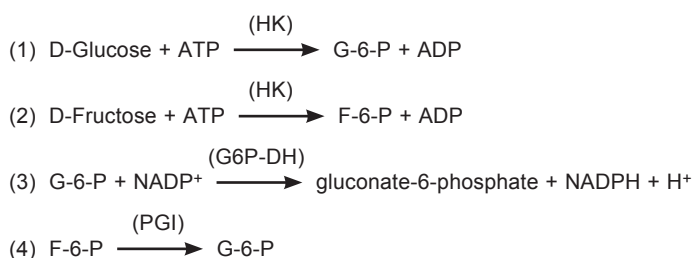
Use:

For the specific measurement of D-fructose and/or D-glucose especially in wines, fruit juices, beverages and food products.

For specific sample preparation methods refer to the D-Fructose/D-Glucose Assay Kit (K-FRUGL) data booklet.

Assay Principle:

Conversion of D-fructose and/or D-glucose via the following reactions is directly proportional to the coupled formation of NADPH:



Procedure:

Prepare the assay reagents and calibrators and use with the K-FRUGL (GLUCOSE) and K-FRUGL (TOTAL) ChemWell®-T assay files and the K-FRUGL (FRUCTOSE) ChemWell®-T indices file.

D-Fructose/D-Glucose Assay Kit Components:

- Bottle 1:** Buffer (15 mL, pH 7.6) plus sodium azide (0.02% w/v) as a preservative.
Stable for > 2 years at 4°C.
- Bottle 2:** NADP⁺, ATP and PVP.
Stable for > 5 years at -20°C.
- Bottle 3:** Hexokinase plus glucose-6-phosphate dehydrogenase suspension (2.25 mL).
Stable for > 2 years at 4°C.
- Bottle 4:** Phosphoglucose isomerase suspension (2.25 mL).
Stable for > 2 years at 4°C.

Preparation of Kit Components:

1. Use the contents of bottle 1 as supplied.
Stable for > 2 years at 4°C.
2. Dissolve the contents of bottle 2 in 12 mL of distilled water. **Stable for > 1 year at 4°C** or > 2 years at -20°C (to avoid repetitive freeze / thaw cycles, divide into appropriately sized aliquots and store in polypropylene tubes).
- 3 & 4. Use the contents of bottles 3 and 4 as supplied.
Swirl the bottle to mix contents before use.
Stable for > 2 years at 4°C.

Preparation of Assay Reagents: (per ~ 105 assays)

Reagent 1:

Component	Volume
distilled water	19 mL
bottle 1 (buffer)	1 mL
*bottle 2 (NADP/ATP)	1 mL
Total volume	21 mL

*after adding 12 mL of distilled water

Reagent 1 stability: > 30 days at 4°C

Reagent 2:

Component	Volume
distilled water	1.9 mL
bottle 3 (HK/G6P-DH)	0.2 mL
Total volume	2.1 mL

Reagent 2 stability: > 30 days at 4°C

Reagent 3:

Component	Volume
distilled water	1.9 mL
bottle 4 (PGI)	0.2 mL
Total volume	2.1 mL

Reagent 3 stability: > 30 days at 4°C

Calibrators:

- K-FRUGL 1: 0 g/L (use distilled water)
K-FRUGL 2: 0.75 g/L of each sugar (D-fructose + D-glucose)
K-FRUGL 3: 1.5 g/L of each sugar (D-fructose + D-glucose)
K-FRUGL 4: 3.0 g/L of each sugar (D-fructose + D-glucose)

Assay Parameters:

Assay volumes: K-FRUGL (GLUCOSE)

Reagent 1: 0.200 mL
Sample: 0.003 mL
Reagent 2: 0.020 mL

Assay volumes: K-FRUGL (TOTAL)

Reagent 1: 0.200 mL
Sample: 0.003 mL
Reagent 2: 0.020 mL
Reagent 3: 0.020 mL

Calibrators: 0, 0.75, 1.5, 3.0 g/L of each sugar (D-fructose + D-glucose)

Reaction time: 5 min at 37°C: K-FRUGL (GLUCOSE)
10 min at 37°C: K-FRUGL (TOTAL)

Wavelength: 340 nm

Assay type: endpoint

Reaction direction: increase

Linearity: up to 6 g/L of total sugar (D-fructose + D-glucose)

ChemWell®-T Files:

- K-FRUGL (GLUCOSE) measures free D-glucose (g/L).
- K-FRUGL (TOTAL) measures free D-glucose plus D-fructose (g/L).
- K-FRUGL (FRUCTOSE) indices file calculates D-fructose (g/L) from K-FRUGL (TOTAL) and K-FRUGL (GLUCOSE).

