



Lactose/D-Galactose (K-LACGAR) Procedure for ChemWell®-T Auto-Analyser

Requirements:

- Lactose/Galactose (Rapid) Assay Kit (K-LACGAR) (provides ~ 1200 assays).
- K-LACGAR (GALACTOSE) and K-LACGAR (TOTAL) ChemWell®-T assay files and the K-LACGAR (LACTOSE) ChemWell®-T indices file.
- Use in association with the Lactose/Galactose (Rapid) Assay Kit (K-LACGAR) product data booklet.

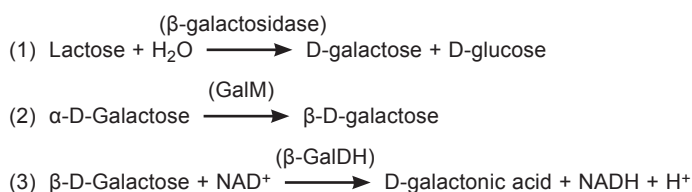
Use:

For the specific measurement of lactose and/or D-galactose especially in dairy, food and plant products.

For specific sample preparation methods refer to the Lactose/Galactose (Rapid) Assay Kit (K-LACGAR) data booklet.

Assay Principle:

Conversion of lactose and/or D-galactose via the following reactions is directly proportional to the coupled formation of NADH:



Procedure:

Prepare the assay reagents and calibrators and use with the K-LACGAR (GALACTOSE) and K-LACGAR (TOTAL) ChemWell®-T assay files and the K-LACGAR (LACTOSE) ChemWell®-T indices file.

Lactose/Galactose (Rapid) Assay Kit Components:

- Bottle 1:** Buffer (2.5 mL, pH 5.0).
Stable for > 2 years at 4°C.
- Bottle 2:** Buffer (25 mL, pH 8.6) plus EDTA and sodium azide (0.02% w/v) as a preservative.
Stable for > 2 years at 4°C.
- Bottle 3:** NAD⁺.
Stable for > 5 years at -20°C.
- Bottle 4:** β-Galactosidase suspension (1.2 mL).
Stable for > 4 years at 4°C.
- Bottle 5:** β-Galactose dehydrogenase plus galactose mutarotase suspension (2.4 mL).
Stable for > 2 years at 4°C.

Preparation of Assay Reagents:

K-LACGAR B1:

Component	Volume
bottle 1 (buffer)	2.5 mL
distilled water	21.5mL
Mix thoroughly then immediately add:	
bottle 4 (β-galactosidase)	1.2 mL
Total volume	25.2 mL

Divide into appropriately sized aliquots and store in polypropylene containers at -20°C between use and keep cool

during use if possible.

K-LACGAR B1 stability: > 2 years at -20°C

K-LACGAR B2:

Use the contents of bottle 2 as supplied.

K-LACGAR B2 stability: > 2 years at 4°C

K-LACGAR B3:

Dissolve the contents of bottle 3 in 12 mL of distilled water.

K-LACGAR B3 stability: > 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).

K-LACGAR B5:

Swirl the bottle to mix contents before use then dilute the required amount of bottle 5 in distilled water at a ratio of 1 part bottle 5 to 9 parts distilled water.

K-LACGAR B5 stability: > 1 days at 4°C

Calibrators:

K-LACGAR 1: 0 g/L (use distilled water)

K-LACGAR 2: 0.213 g/L D-galactose

K-LACGAR 3: 0.425 g/L D-galactose

K-LACGAR 4: 0.85 g/L D-galactose

Assay Parameters:

Assay volumes: **K-LACGAR (GALACTOSE)**

Distilled water: 0.220 mL

Sample: 0.020 mL

K-LACGAR B2: 0.020 mL

K-LACGAR B3: 0.010 mL

K-LACGAR B5: 0.020 mL

Assay volumes: **K-LACGAR (TOTAL)**

K-LACGAR B1: 0.020 mL

Sample: 0.020 mL

Distilled water: 0.200 mL

K-LACGAR B2: 0.020 mL

K-LACGAR B3: 0.010 mL

K-LACGAR B5: 0.020 mL

Calibrators: 0, 0.213, 0.425, 0.85 g/L D-galactose

Reaction time: 5 min at 37°C: K-LACGAR (GALACTOSE)

10 min + 5 min at 37°C: K-LACGAR (TOTAL)

Wavelength: 340 nm

Assay type: endpoint

Reaction direction: increase

Linearity: up to 0.85 g/L D-galactose or 1.6 g/L lactose

ChemWell®-T Files:

- K-LACGAR (GALACTOSE) measures free D-galactose (g/L).
- K-LACGAR (TOTAL) measures free D-galactose plus D-galactose released from lactose (g/L).
- K-LACGAR (LACTOSE) indices file calculates lactose (g/L) from K-LACGAR (TOTAL) and K-LACGAR (GALACTOSE).

