



D-/L-Lactic Acid (K-DLATE) Procedure for ChemWell®-T Auto-Analyser

Requirements:

- D-/L-Lactic Acid (D-/L-Lactate) (Rapid) Assay Kit (K-DLATE) (provides ~ 475 assays).
- K-DLATE (TOTAL) ChemWell®-T assay file.
- Use in association with the D-/L-Lactic Acid (D-/L-Lactate) (Rapid) Assay Kit (K-DLATE) product data booklet.

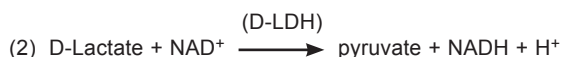
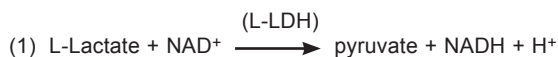
Use:

For the specific measurement of D-/L-lactic acid especially in wines, fruit juices, beverages and food products.

For specific sample preparation methods refer to the D-/L-Lactic Acid (D-/L-Lactate) (Rapid) Assay Kit (K-DLATE) data booklet.

Assay Principle:

Conversion of D-/L-lactic acid 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-DLATE (TOTAL) ChemWell®-T assay file.

D-/L-Lactic Acid (D-/L-Lactate) (Rapid) Assay Kit Components:

Bottle 1: (x2) Buffer (25 mL, pH 10.0) plus D-glutamate and sodium azide (0.02% w/v) as a preservative.
Stable for > 2 years at 4°C.

Bottle 2: (x2) NAD⁺.
Stable for > 5 years at -20°C.

Bottle 3: D-Glutamate-pyruvate transaminase suspension (2.2 mL).
Stable for > 2 years at 4°C.

Bottle 4: L-Lactate dehydrogenase suspension (1.1 mL).
Stable for > 2 years at 4°C.

Bottle 5: D-Lactate dehydrogenase suspension (1.1 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 5.5 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 & 5. Use the contents of bottles 3, 4 and 5 as supplied.
Swirl the bottle to mix contents before use.
Stable for > 2 years at 4°C.

Preparation of Assay Reagents: (per ~ 95 assays)

Reagent 1:

| Component | Volume |
|-------------------|---------|
| distilled water | 12.9 mL |
| bottle 1 (buffer) | 5 mL |
| *bottle 2 (NAD) | 1 mL |
| bottle 3 (D-GPT) | 0.2 mL |
| Total volume | 19.1 mL |

*after adding 5.5 mL of distilled water

Reagent 1 stability: > 2 days at 4°C

Reagent 2:

| Component | Volume |
|------------------|--------|
| distilled water | 2.0 mL |
| bottle 4 (L-LDH) | 0.2 mL |
| bottle 5 (D-LDH) | 0.2 mL |
| Total volume | 2.4 mL |

Reagent 2 stability: > 2 days at 4°C

Calibrators:

- K-DLATE 1: 0 g/L (use distilled water)
K-DLATE 2: 0.25 g/L D-/L-lactic acid
K-DLATE 3: 0.5 g/L D-/L-lactic acid
K-DLATE 4: 1.0 g/L D-/L-lactic acid

Assay Parameters:

- Assay volumes: Reagent 1: 0.200 mL
Sample: 0.010 mL
Reagent 2: 0.025 mL
- Calibrators: 0, 0.25, 0.5, 1.0 g/L D-/L-lactic acid
Reaction time: 10 min at 37°C
Wavelength: 340 nm
Assay type: endpoint
Reaction direction: increase
Linearity: up to 1 g/L of D-/L-lactic acid

Note:

For the measurement of L-lactic acid only, use the L-Lactic Acid (K-DLATE) procedure with the associated K-DLATE (L) ChemWell®-T assay file.

For the measurement of D-lactic acid only, use the D-Lactic Acid (K-DLATE) procedure with the associated K-DLATE (D) ChemWell®-T assay file.

