



# L-Malic Acid (K-LMALAF) Procedure for ChemWell®-T Auto-Analyser

## Requirements:

- L-Malic Acid (Analyser Format) Assay Kit (K-LMALAF) (provides ~ 1116 assays).
- K-LMALAF ChemWell®-T assay file.
- Use in association with the L-Malic Acid (Analyser Format) Assay Kit (K-LMALAF) product data booklet.

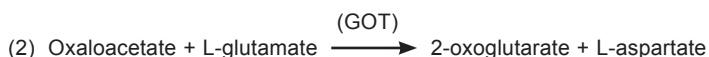
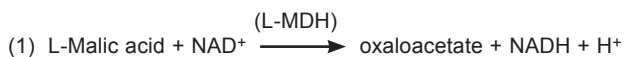
## Use:

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

For specific sample preparation methods refer to the L-Malic Acid (Analyser Format) Assay Kit (K-LMALAF) data booklet.

## Assay Principle:

Conversion of L-malic 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-LMALAF ChemWell®-T assay file.

## L-Malic Acid (Analyser Format) Assay Kit Components:

**Bottle 1:** Buffer (48 mL, pH 10.0) plus L-glutamate and sodium azide (0.02% w/v) as a preservative. Stable for > 2 years at 4°C.

**Bottle 2: (x2)** NAD<sup>+</sup> and PVP. Freeze dried powder. Stable for > 5 years at -20°C.

**Bottle 3:** Glutamate-oxaloacetate transaminase suspension (2.5 mL). Stable for > 2 years at 4°C.

**Bottle 4:** L-Malate dehydrogenase suspension (2.5 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 ~ 93 assays)

### Reagent 1:

Component	Volume
distilled water	12.4 mL
bottle 1 (buffer)	4 mL
*bottle 2 (NAD/PVP)	2 mL
bottle 3 (GOT)	0.2 mL
Total volume	18.6 mL

\*after adding 12 mL of distilled water

Reagent 1 stability: > 7 days at 4°C

### Reagent 2:

Component	Volume
distilled water	2.1 mL
bottle 4 (L-MDH)	0.2 mL
Total volume	2.3 mL

Reagent 2 stability: > 7 days at 4°C

## Calibrators:

- K-LMALAF 1: 0 g/L (use distilled water)
- K-LMALAF 2: 1.5 g/L L-malic acid
- K-LMALAF 3: 3.0 g/L L-malic acid
- K-LMALAF 4: 6.0 g/L L-malic acid

## Assay Parameters:

- Assay volumes: Reagent 1: 0.200 mL  
Sample: 0.003 mL  
Reagent 2: 0.020 mL
- Calibrators: 0, 1.5, 3.0, 6.0 g/L L-malic acid
- Reaction time: 3 min at 37°C
- Wavelength: 340 nm
- Assay type: endpoint
- Reaction direction: increase
- Linearity: up to 6 g/L of L-malic acid

