

Megazyme

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L-MALIC ACID (Analyser Format)

ASSAY PROCEDURE FOR AUTO-ANALYSER APPLICATIONS

K-LMALAF 08/18

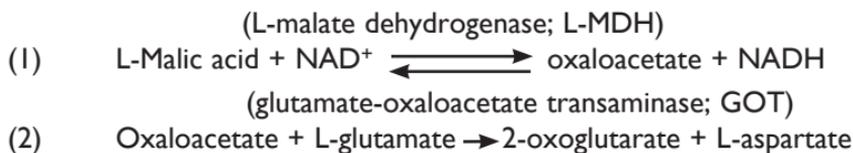
[245.5 mL of reagent (R1 + R2) per kit;
equivalent to 1116 reactions of 0.22 mL]



INTRODUCTION:

Some kits sold for manual enzymatic analysis of L-malic acid can't be used effectively in an auto-analyser format, as they either lack linearity at higher substrate concentrations, on-machine stability, or the reagents are not present in optimal ratios, leading to significant wastage and thus increased cost per analysis. This kit has, however, been specifically developed for auto-analyser applications, and offers linearity up to 80 µg/mL of L-malic acid (in the final reaction solution), excellent on-machine stability and contains optimal amounts of each reagent. Polyvinylpyrrolidone (PVP) has also been incorporated into the assay format to prevent performance inhibition by polyphenolic compounds (tannins). At the same time, the biochemical principle behind this assay format remains the same as all other L-malic acid kits (see equations 1 and 2 below).

PRINCIPLE:



SAFETY:

The general safety measures that apply to all chemical substances should be adhered to.

For more information regarding the safe usage and handling of this product please refer to the associated SDS that is available from the Megazyme website.

KITS:

Kits suitable for the preparation of 245.5 mL of reagent (equivalent to 1116 reactions of 0.22 mL) are available from Megazyme. The kits contain the full assay method plus:

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⁺ plus PVP. Stable for > 5 years below -10°C (freeze dried powder). Dissolve the contents of one of bottle 2 in 12 mL of distilled water. **Stable for > 1 year at 4°C** or stable for > 2 years below -10°C (to avoid repetitive freeze/thaw cycles, divide into appropriately sized aliquots and store in polypropylene tubes). **Do not** dissolve the contents of the second bottle until required.

- 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.
- Bottle 5:** L-Malic Acid Standard (2 mL) (6 g/L). Ready to use.
Stable for > 2 years at 4°C.

REAGENT PREPARATION:

Preparation of R1:

Component	Volume
bottle 1 (buffer)	4.0 mL
bottle 2 (NAD ⁺ /PVP)	2.0 mL (after adding 12 mL of H ₂ O to bottle 2)
bottle 3 (GOT)	0.2 mL (swirl to mix before use)
H ₂ O	12.4 mL
Total volume	18.6 mL

Preparation of R2:

Component	Volume
bottle 4 (L-MDH)	0.2 mL (swirl to mix before use)
H ₂ O	2.1 mL
Total volume	2.3 mL

EXAMPLE METHOD:

R1: 0.200 mL

Sample: ~ 0.002 mL

(allow R1 and sample to incubate for 3 min before addition of R2).

R2: 0.020 mL

Reaction time: 3 min at either 20-25°C or 37°C

Wavelength: 340 nm

Prepared reagent stability: > 7 days when refrigerated

Calculation: endpoint

Reaction direction: increase

Linearity: up to 80 µg/mL of L-malic acid in final reaction solution

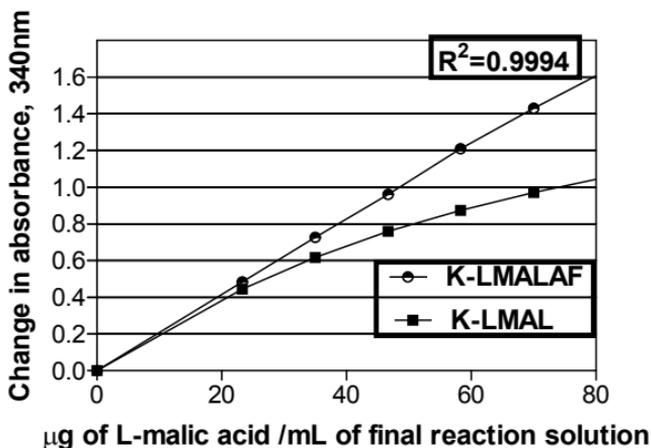


Figure 1. Calibration curve showing extended linearity of **K-LMALAF** as compared to **K-LMAL**. The reactions used to generate these calibration curves were performed at 25°C for 3 min, using a 4.6 mm path-length cuvette.

Although **K-LMAL** exhibits linearity beyond that expected from manual analysis (~ 13 μg/mL), the much higher concentrations demanded by the auto-analyst can only be achieved using **K-LMALAF**.



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