Test comparison for D-Malic acid between Roche and Enzytec™ *Liquid*

Art. No. E8270

Overview

Item	Roche	Enzytec™ *Liquid*
Art. No.	11 215 558 035	E8270
Kit size	3 x 11 tests	50 tests
Test principle	D-Malic acid is oxidized to oxaloacetate by D-malate-dehydrogenase (D-MDH). Oxaloacetate is immediately split by the same enzyme to pyruvate and CO_2: D-Malate + NAD^+ — D-MDH —> pyruvate + CO_2 + NADH + H^+	
Calculation of results	The method is an end-point reaction where results are calculated using the Lambert-Beer formula (excel table available on request)	
Registration	This method is registered by the AIJN/IFU (fruit juices), DIN (Germany), EN (European norm), OIV (wine)	
Reagents	• Vial 1 = Buffer, glutamate (liquid) • Vial 2 = NAD (lyophilizate) • Vial 3 = D-MDH (lyophilizate)	• R1 = Buffer • R2 = NAD, D-MDH
Stability after reconstitution	• Vial 2 = 3 weeks • Vial 3 = 5 days	All reagents are liquid, ready-to-use and stable after opening
QC samples	QC included (vial 4)	QC must be purchased separately
Procedure	**Steps** / **Samples (or blank)** Vial 1 / 1.000 mL Vial 2 / 0.200 mL Sample (or H_2O) / 0.100 mL Water / 1.700 mL Incub. 6 min, read A_1 Vial 3 / 0.050 mL Incub. 20 min, read A_2	**Steps** / **Samples (or blank)** R1 / 2.000 mL Sample (or H_2O) / 0.100 mL Incub. 3 min, read A_1 R2 / 0.500 mL Incub. 15 min, read A_2
Linearity	→ 500 mg/L (sample = 0.100 mL)	→ 500 mg/L (sample = 0.100 mL)
Calculation with Lambert-Beer: • LoD (v = 1 mL, ΔA = 0.010) • LoQ (v = 1 mL, ΔA = 0.050)	• 0.65 mg/L • 3.15 mg/L	• 0.7 mg/L • 3.7 mg/L
	The real LoD and LoQ have been measured only for Enzytec™ *Liquid* E8270 using the method DIN 32645:2008-11 with 100 µL sample: LoD = 10 mg/L and LoQ = 30 mg/L. By increasing the sample volume to 1 mL, these values can be reduced by factor 10.	

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Evaluation Enzytec™ *Liquid* D-Malic acid (Art. No. E8270) versus Roche

a) Evaluation program for individual labs

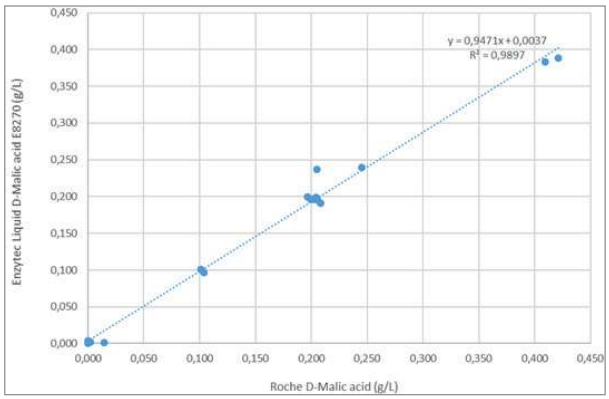
We suggest following evaluation program:

- Purchase one kit Enzytec™ *Liquid* D-Malic acid (E8270, 50 tests) and run in parallel with the Roche kit
- For testing the recovery, always run a QC sample (to be purchased separately)
- If available, run a QC sample specific for the laboratory and calculate the recovery
- Run the routine samples in parallel with both kits until the 50 tests are finished (method comparison)
- Keep the same sample preparation as for the Roche kit and use the same vial containing the sample solution for both tests (100 µL each)
- Perform the two assays at the same time to avoid problems with sample stability (especially for Acetaldehyde, Acetic acid, Ammonia, Ascorbic acid, Ethanol and Sulfite which are unstable)

It is possible to test more validation criteria (e.g. linearity, precision or reproducibility), but more kits will be necessary.

b) Results of internal evaluation at R-Biopharm

- Recovery of QC samples
  Recovery is 100 ± 5 %
  Note: this is part of the specifications for all Enzytec™ *Liquid* test kits and is checked for every lot produced
- Method comparison



This method comparison was performed with different control materials and different types of fruit juices (orange juice, multi-fruit juice, apple juice). All points are well aligned along the equivalence line (y = x) and the coefficient of correlation (R^2) is 0.99.