Inspiring innovation.

L-MALIC ACID ENZYMATIC PROCEDURE

Equipment

- Spectrophotometer UV, set to 340nm
- Cuvettes, UV disposable (10 mm pathlength)
- *Repetitive syringe dispenser (Nichiryo Model 8100)
  - 60 mL syringe
  - 3 mL syringe
  - 0.6 mL syringe (x2)
- *25 µL microdispenser (fixed volume, positive displacement)
- Parafilm
- Cuvette tray (empty box from disposable cuvettes or cuvette rack)
- Timer

Reagents

- Malic Acid buffer(1)
- NAD(2) 1000 mg, dissolved in 24mL DI Water(1)
- GOT(2) 200 IU/mg, suspension of 2 mg/mL
- Malate Dehydrogenase(2) 1200 IU/mg, solution of 5 mg/mL
- DI Water
- Malic Acid Standards(1):

  100mg L( )Malic acid/100mL DI Water

  400mg L( )Malic acid/100mL DI Water

1. Prepared by by Enartis USA
2. Purchased from Roche Diagnostics

Procedure

Samples are analyzed in batches (a.k.a. "runs"). Samples are run singly and are accompanied by a blank and two check standards.

1. Make the normal entries in the log book for the blank, the standards, and the samples.
2. Turn on spectrophotometer and allow to warm up at least 5 minutes.

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3. Place one cuvette for the blank, each standard, and each sample into the tray.
4. Fill cuvettes with 3 mLs of Buffer solution using repetitive syringe dispenser.
5. Add 100 µL of NAD to each cuvette using repetitive syringe dispenser.
6. Add 10 µL of GOT to each cuvette using repetitive syringe dispenser.
7. Add 25 µL of DI Water to blank cuvette, 25 µL of Malic Acid Standard to standard cuvettes, and 25 µL of sample to each of the remaining cuvettes using the 25 µL fixed volume microdispenser.
8. Cover cuvette with Parafilm and mix by inverting 3 times. Zero spectrophotometer on water and record initial absorbance (A_1) of each cuvette.
9. Add 10 µL of Malate Dehydrogenase to each cuvette using repetitive syringe dispenser. Mix as above and incubate for 10 minutes at room temperature.
10. Read and record final absorbance (A_2) of each cuvette.

Calculations

$\Delta A = (A_2\text{-}A_1)_{sample} - (A_2\text{-}A_1)_{blank}$

Malic Acid [mg/100mL] $= \Delta A \times [(V \times MW)/(\epsilon \times d \times s \times 10)]$

$= \Delta A \times 267.75$

Where:

V = 3.145 mL	final volume
s = 0.025 mL	sample volume [mL]
MW = 134.09 g/mol	molecular of Malic Acid (substance assayed)
d = 1.0 cm	light path [cm]
ϵ = 6.3 $Lxmmol^{-1}xcm^{-1}$	extinction coefficient of NADH at 340 nm

Notes

- The range of this assay is 0 to 400 mg/100mL. Samples with higher levels must be diluted.
- Buffer must be at room temperature for assay.
- Buffer is stable approximately 60 days refrigerated.
- Do not change enzymes midway through a run. Open a new bottle, enough to put in all cuvettes, before beginning procedure.
- Color does not interfere.
- Malic Acid Standards should be replaced at least once a month but should be stable for two weeks if refrigerated.
- See Roche instruction sheets for more information.
- *Eppendorf pipettors can be used in place of syringe dispenser and microdispenser. 3 mL volume can be dispensed with Repipet Jr dispenser.
- A value less than 30 mg/100mL is considered Bottle Stable.

Disposal

Liquids in sink and solids in trash.

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