

Enzymatic measurement of **MALATE** content

<Reagents>

1) Assay buffer ; Hydrazine and glycine buffer (pH 9.0@25°C)

	Hydrazine	glycine
preparation	8.34 mL	3.8 g / 70 mL

Adjust pH 9.0 and fill up to 100 mL

2) NAD solution

	NAD
preparation	50 mg / mL
for 40 spls	50 mg / mL $\times 2$
for 60 spls	50 mg / mL $\times 3$
for 80 spls	50 mg / mL $\times 4$
for 100 spls	50 mg / mL $\times 5$

3) MDH solution

	MDH	Milli-Q
for 40 spls	26 μ L	62 μ L
for 60 spls	40 μ L	92 μ L
for 80 spls	53 μ L	123 μ L
for 100 spls	66 μ L	154 μ L

4) Malate solution for STD

	1 mM malate
preparation	6.7 mg / 50 mL

malate concentration (μ M)	0	5	10	20	50	100
malate content (nmol)	0	3.4	6.8	13.6	34	68
1mM malate (μ L)	0	3.4	6.8	13.6	34	68
Milli-Q (μ L)	680	677	673	666	646	612

<Procedure>

1. Mix the following solutions in each cuvette (for UV)

0.38 mL Milli-Q
0.3 mL sample
0.75 mL assay buffer

2. Add 50 μ L NAD solution in 30 sec intervals and mix well

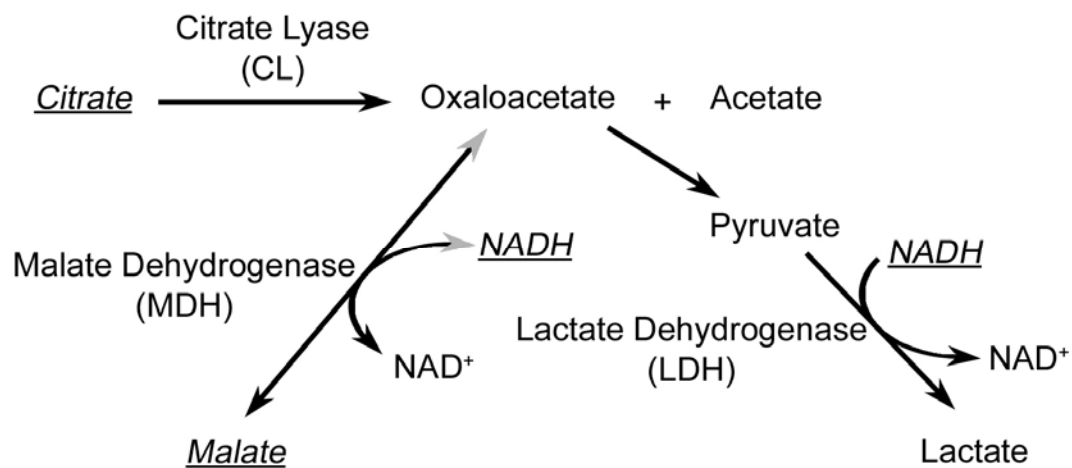
3. Incubate 60 min at room temperature

4. Read A_{340} in 30 sec intervals

5. Add 2 μ L MDH solution in 30 sec intervals and mix well

6. Incubate 60 min at room temperature

7. Read A_{340} in 30 sec intervals



Delhaize E, Ryan P.R., Randall P.J. (1993) Aluminum tolerance in wheat (*Triticum aestivum* L.). II. Aluminum-stimulated excretion of malic acid from root apices. *Plant Physiol.* **103**, 695 - 702.