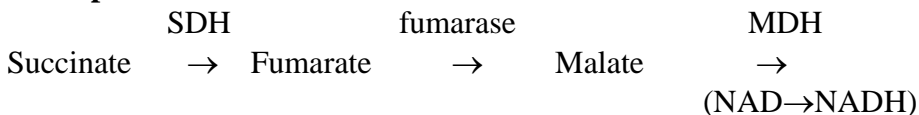


Succinate Dehydrogenase

References-

1) Lowry et al : Effects of detraining on enzymes of energy metabolism.. Am. J. Physiology 244(Cell Physiology 13):C276-C287

Principle-



GOT



Solutions- make the following stock solutions-

1. **1M Imidazole HCl pH 7.4** – Imidazole Sigma I-0250 MW 68.08. Weigh 6.80 gms Imidazole. Dissolve in approximately 90 mls MilliQ water. Adjust ph to 7.4 with 12N HCl. Adjust final volume to 100 mls. The solution can be kept for up to 1 year at 4°C. Discard if solution appears turbid.
2. **1M Succinate-** Sigma S5047 FW 270.1. Weigh 6.75 gms/25 ml.Store in 5 ml aliquots at -80°C for up to 1 year.
3. **0.5M K₃FeCN₆-** Sigma P8131 FW 329.2 Weigh 8.23 gms, dissolve in final volume of 50 mls. Store at 4°C for up to 1 year.
4. **10 mM Fumarate.** Sigma F1506 MW 160. Weigh 40 mg per final volume 25 mls of Milliq Water.
5. **1M 2-Amino-2 Methyl 1,3- Propanediol pH 8.8-** Sigma A9074 FW 105.1 Weigh 10.51 gms/ final volume 100 mls. ph adjusted to 8.8 with 12N HCl.
6. **1M Glutamate** –Sigma G 1626 FW 169.1 Weigh 1.69 gms, dissolve in final volume 10 mls. Store at - 80°C for up to 1 year.
7. **100 mM NAD Sigma N1636 FW 66.34** Weigh 100 mgs, Dissolve in 1.5 mls. Store at -80°C for up to 1 year.

Assay Reagent-

Make fresh on day of assay-

Final conc.	Stock conc.	Lot No.	Volume/10 ml
50 mM Imid HCl	1M		0.5 ml
100 mM Succinate	1M		1.0 ml
10 mM K ₃ FeCN ₆	500 mM		0.2 ml
0.02% BSA	1%		0.2 mls

Protocol-

Add 100 µL Assay Reagent to tubes. Add 10µL Medium to blanks, and 10µL 1:10 homogenate to sample tubes. Incubate at RT for 60 mins. Add 10µL 1N NaOH, and heat 20 minutes at 60°C. Add 1 ml Fumarate Indicator* reagent, and read at 340 nM in The UNICO Spectrophotometer, using disposable semi micro cuvettes (OD1). (Add standards at this step.)

Add 5 µg MDH(5mg/ml) stock, use 2 µL. Read at 20 minutes.(OD2)

#	AR	Med	1:10 Homog	OD1	OD2	Net OD
1.						
2.						
3.						
4.						
5.						
6.						
7.						
8.						
9.						
10.						
11.						
12.						

***Fumarate Indicator-**

Final conc. ml	Stock conc.	Volume/20
50 mM 2-Amino-2 methyl propaneidol ph8.8	1M	1 ml
200µM NAD	100mM	40µL
10mM glutamate	1M	20µL
5µg/ml pig heart fumarase (E.C. 4.2.1.2)	6.3 mg/ml	16µL
2µg/ml GOT (E.C. 2.6.1.1)	7.8 mg/ml	5µL

Standards- 100 µL Assay Reagent + 10 µL 1N NaOH + 1 ml Fumarate Indicator.

	OD1	MDH	OD2
1. Blank			
2. 2.5µL 10 mM Fumarate			
3. 5µL 10 mM Fumarate			
4. 10µL 10 mM Fumarate			

$$\mu\text{moles/min/gm} = \frac{175 \text{ nmoles std} \times \text{Final volume} \times \text{sample od}}{\text{std od} \times 1 \text{ mg} \times 60 \text{ min}}$$

Updated 2.27.09 RC

Approved Dr. Alan Pestronk