

## Citrate Synthase Activity Assay

modified from Neuromuscular Clinical Laboratory, Neurology, WUMS

Principle:



Acetyl CoA has an absorption band at 232 nM due to the thioester bond. As the assay proceeds and the Acetyl CoA is used, there is a decrease in absorption.

Acetyl CoA:  $E = 5.4 \text{ cm}^2/\mu\text{mole}$

### Solutions: (stock solutions)

- 1) **1M Tris HCl, pH 7.4** (for 100 mls final volume)  
Tris HCl (Sigma T-7149, MW=157.6). Store RT 15.76 g
- 2) **3M KCl** (for 100 mls final volume)  
KCl (Sigma P3911, MW=74.55). Store RT 22.36 g
- 3) **1M Tris HCl, pH 8.1** (for 50 mls final volume)  
Trizma Base (Sigma T-1503, MW=121.1) 3.028 g  
Tris HCl (Sigma T-7149, MW=157.6) 3.94 g  
pH to 8.1 with either 1N HCl or NaOH

Can store at -20°C until use. Thaw and keep on ice

- 4) **0.5M Oxaloacetic Acid** (Sigma O-4126, MW=132.1)  
Dissolve 132 mgs in 2mls ddH<sub>2</sub>O. Store at -80°C

- 5) **0.2M Acetyl CoA** (Sigma A-2181, MW=809.6)  
Use entire bottle (100mgs), add 617.5  $\mu\text{l}$ s ddH<sub>2</sub>O.

Store at -80°C. Keep on ice when thawed.

### Solutions: (make fresh)

#### **1. Homogenization Medium (HM):**

| Final Conc.       | Stock Conc. | Volume / 25 ml |
|-------------------|-------------|----------------|
| 50mM Tris, pH 7.4 | 1M          | 1.25           |
| 0.15M KCl         | 3M          | 1.25           |

#### **2. Assay Reagent (AR):**

| Final Conc. | Stock Conc. | Volume / 10 ml    |
|-------------|-------------|-------------------|
| 100mM Tris  | 1M          | 1.0 ml            |
| 0.17mM OAA  | 0.5M        | 3.4 $\mu\text{l}$ |
| 0.2mM AcCoA | 0.2M        | 10 $\mu\text{l}$  |

### Tissue Preparation:

For skeletal muscle, weigh tissue sample (~20-50 mg), place into pre-chilled glass homogenizer and add 9 volumes of HM. Hand homogenize, then transfer to 1.5 ml centrifuge tube. Spin for 10 minutes at 1000g (~4000rpm) at 4°C in microcentrifuge in the cold room. Remove supernatant to a new tube and freeze in liquid nitrogen. Store at -80°C until later use. For heart, activity will likely be too high. I suggest that a 1:20 dilution is used.

### Assay:

- Assay should be run with a blank for every time point.
- Thaw samples, keep on ice. Allow AR to equilibrate to room temperature. Note temp. and ensure any repeats are performed at a similar temperature.
- Set Spec to read at 232 nm.
- Place 400 µl AR into each cuvette-no more than 5 total at a time (1 per sample + 1 blank).
- 2 µl of HM or sample into appropriate cuvette and quickly load. Set timer after first addition.
- Read sample at 1', 2', 5' & 8'.
- 1' reading synchronizes samples, data analysis utilizes 2', 5' & 8' readings.
- Calculate enzyme activity from OD as follows:
  - Convert OD to whole number (multiply by 1000)
  - Determine delta OD from 2'-5' & from 2'-8' for each cuvette (column A)
  - Determine rate of change (OD/min). Note this is the delta for your sample cuvette corrected for drift of blank.
    - 2'-5' (B6) = (A5-A2)/3
    - 2'-8' (C6) = (A6-A3)/6
  - Convert to  $\mu\text{moles} \cdot \text{gm}^{-1} \cdot \text{min}^{-1} = (\text{net OD}/\text{min} \cdot 1000 \cdot 1.005) / (5440 \cdot 0.2)$   
(1.005 = for tissue volume; 0.2 for mgs in assay)

|   | Sample |    | OD    | A   | B     | C     | D            | E            |
|---|--------|----|-------|-----|-------|-------|--------------|--------------|
| 1 | Blank  | 2' | 709   |     |       |       |              |              |
| 2 | Blank  | 5' | 706   | 3   |       |       |              |              |
| 3 | Blank  | 8' | 707.5 | 1.5 |       |       |              |              |
| 4 | 9-24   | 2' | 868.5 |     |       |       |              |              |
| 5 | 9-24   | 5' | 653.5 | 215 |       |       |              |              |
| 6 | 9-24   | 8' | 583.5 | 285 | 70.67 | 47.25 | <b>65.28</b> | <b>43.65</b> |

- Note that the value for 2' to 8' (E6) is much lower than that for 2' to 5' (D6) in the example. Substrate (Acetyl CoA) can become limiting in this assay with highly active tissue, leading to reduction of slope. If this is the case, use 1 µl of sample instead of 2 µl (and change formula to 0.1 for mgs in assay).