Summary

Acetyl CoA synthase catalyzes the conversion of acetate, ATP and coenzyme A into acetyl CoA and AMP. This assay involves the chemical reaction between acetyl CoA and hydroxylamine which liberates CoASH and hydroxamic acid which can be measured colorimetrically.

Solutions Required

1. 200 mM Tris HCl buffer (FW 157.6)
   (3.15 g/100 mL solution)
   adjust to a pH of 8.1 with 20% KOH
   can be stored in stock

2. 100 mM MgCl2·6H20 (FW 203.3)
   (2.03 g/100 mL solution)
   can be stored in stock

3. 400 mM sodium acetate trihydrate (FW 138.08)
   (5.44 g/100 mL solution)
   can be prepared in stock solution, stored in refrigerator.

4. 3.33 mM CoA Lithium salt (FW 767.5)
   (2.56 mg/mL solution)
   Sigma catalog #C3019
   must be prepared fresh

5. 100 mM ATP disodium salt (FW 551.5)
   (55.15 mg/mL solution)
   Sigma catalog #A2383
   must be prepared fresh

6. 2.5 g FeCl3 in 100 mL 2.0M HCl
   can be stored in stock

7. 1.0M hydroxylamine HCl (FW 69.49)
   mix equal volumes of 2M NH2OH·HCl (139 mg/mL) and 2M KOH (56.1 mg/mL)
   Sigma catalog #H2391
   must be prepared fresh (or frozen stock)

8. 10 mM lithium potassium acetyl phosphate (FW 184.1)
   (1.84 mg/mL solution)
   Sigma catalog #A0262
   must be prepared fresh
9. 1M KF (FW 58.10)  
   (58.10 mg/mL solution)  
   Sigma catalog #60238  
   can be stored in stock

10. 200 mM glutathione (reduced) neutralized with KOH to pH 4.5  
    (61.46 mg/mL solution)  
    Sigma catalog #G6529  
    can be prepared in stock solution, stored in refrigerator.

Preparation of Cell Extract

   Follow general protocol **Preparation of Cell Extract**.

1. After first pelletization of cells, resuspend at 4°C in 20 mM Tris HCl pH 8.1 with 20% glycerol.
2. After second pelletization of cells, resuspend at 4°C in 20 mM Tris HCl pH 8.1 with 20% glycerol.

Procedure

1. For each solution to be assayed, prepare the following solutions in a small test tube:

<table>
<thead>
<tr>
<th>Solution</th>
<th>Volume (µL)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris HCl</td>
<td>250</td>
<td>250</td>
</tr>
<tr>
<td>hydroxylamine</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>sodium acetate</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>coenzyme A</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>ATP</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>water</td>
<td>150</td>
<td>250</td>
</tr>
<tr>
<td>glutathione</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>KF</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>

2. Prepare the following lithium acetyl phosphate (LAP) standards:

<table>
<thead>
<tr>
<th>Standard Concentration</th>
<th>DI water</th>
<th>Volume (µL)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0 µmol/mL</td>
<td>450</td>
<td>200</td>
<td>100</td>
<td>250</td>
<td></td>
</tr>
<tr>
<td>1.0 µmol/mL</td>
<td>550</td>
<td>100</td>
<td>100</td>
<td>250</td>
<td></td>
</tr>
<tr>
<td>0.6 µmol/mL</td>
<td>590</td>
<td>60</td>
<td>100</td>
<td>250</td>
<td></td>
</tr>
<tr>
<td>0.3 µmol/mL</td>
<td>620</td>
<td>30</td>
<td>100</td>
<td>250</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>650</td>
<td>0</td>
<td>100</td>
<td>250</td>
<td></td>
</tr>
</tbody>
</table>
3. Place the solutions to be assayed (not the standards) in a water bath at 37°C for 5 minutes.
4. Initiate the reactions by adding 100 µL cell-free extract into each of the test tubes and agitating.
5. Permit the reactions to occur for 20 minutes in the water bath.
6. After 20 minutes, terminate the reaction by adding 1000 µL acidic FeCl₃ solution. At this time, also mix the acidic FeCl₃ solution with the standard solutions.
7. Centrifuge terminated reaction for at 13000 rpm for 10 minutes to pellet precipitated protein
8. Measure the absorbance of each solution in a spectrophotometer at 520 nm.

† Volume of cell extract may be adjusted. Any change in cell extract should be accompanied by a change in the DI water in the cocktails. (For example, if 50 µL of cell extract were used in the experimental, then 300 µL of DI water would be used.) If a sample volume below 50 µL is required to obtain a result within range of the standards, the sample should be diluted prior to mixing with the reagents. For example, in order to dilute by a factor of 10, combine 900 µL of DI water with 100 µL of sample. Then use 100 µL of this diluted solution for the enzyme assay.

Calculation of Activity

One unit (U) of acetyl CoA synthase activity is defined as the amount of enzyme required to produce 1.0 µmole of acetyl CoA in one minute.

1. Fit the results of the standards to the equation: Conc (µmol/mL) = m × Abs

2. Activity = \( \frac{1000 \times TV \times m \times Abs \times D}{t_R \times V \times CF} \)

Activity: Volumetric Activity (U/L)
Abs: Absorbance of sample at 520 nm relative to blank.
m: The best-fitting slope.
TV: Total volume in cuvette (1000 µL)
V: Volume of cell extract used (100 µL)
t_R: Time of reaction (20 minutes)
CF: Concentration factor of cell extract (e.g., if a 100 mL sample is concentrated to a 5 mL volume for the French Press, then CF=20)
D: Dilution factor for cell extract used in the reaction mixture (e.g., if the cell extract is diluted by a factor of 10 prior to being mixed with the reagents, then D = 10)

3. Specific Activity = \( \frac{Activity}{Protein \ Concentration} \)

Activity: Volumetric Activity, as calculated in #2 above (U/L)
Protein Concentration: Protein concentration, as calculated in protocol Total Protein Concentration (mg/L)
Specific Activity: (U/mg protein)
References
