

Response to Editorial comments:

1. There are many long paragraphs as 'Notes' within the protocol. Please either make these into numbered protocol steps (in the imperative; no more than 4 sentences per step) or move them to the Introduction, Results, or Discussion as appropriate.

Response: Thanks for the suggestion. We have moved the long paragraphs as 'Notes' to the discussion section.

2. The second activity equation ($\text{reaction rate} \times 1000 / 13.6 \times \text{path length}$) looks like it should produce units of $\mu\text{mol}/\text{min}/\text{L}$ (i.e., $\mu\text{M}/\text{min}$); it is also unclear how this is 'activity per enzyme activity unit'. Please clarify.

Response: Thanks for your comment. Some commercial kits recommend the citrate synthase activity of samples to be expressed as enzyme activity units per microliter of sample (U/ml) or enzyme activity units per microgram of protein (U/ μg). One unit of citrate synthase is the amount of enzyme that will generate 1.0 mol CoA per minute at pH 7.2 at 25 °C. $1000/13.6 \times \text{path length}$ is only a constant, which does not affect the relative relationships of samples. In this protocol, we only calculated the relative citrate synthase activities by the first activity equation, which is in step 7. Therefore, in the revised version, we deleted the second activity equation in the note of step 7, which may confuse readers.

3. The linear fits in Figure 1 are confusing-it looks like they're only fit to the first few points (i.e., the linear/steady-state portion of the graph), but extend well beyond those points. Can you clarify this; e.g., by shortening the lines?

Response: We agree. Thanks for the suggestion. We shortened the lines in Figure 1.