

TROUBLESHOOTING ENZYME ASSAYS

Greg Crowther, UW Dept. of Medicine

Last updated on May 29, 2009

I. Reagent stocks

- A. Are you using the intended ingredients? (For example, do you need the reduced form of NAD, or the oxidized form? Is your enzyme adenylosuccinate lyase or adenylosuccinate synthetase? Does the label on the container make it clear what's inside?)
- B. Is this particular batch/variety of the reagent known to give good results, or have we not tried it until now? (Example: switching from yeast pyrophosphatase to *E. coli* pyrophosphatase.)
- C. Is the reagent degraded? (Has it been left out overnight at room temperature when it needs to be stored in the fridge or freezer? Does Sigma package it with a special stabilizing gas that leaks out when you open the vial for the first time?)
- D. Is the concentration of the stock appropriate for this experiment? (Again, what does the label say? Can you trust the person who made the stock?)

II. Procedural issues

- A. Have you successfully done this experiment before?
 1. If not, who has done it before?
 - a. Someone in the lab -- check with them directly.
 - b. Someone outside the lab -- check the published protocol/notes.
 - c. No one -- good luck.... Remember that some recombinant enzymes may simply be inactive.
 2. If so, what are the differences between the previous experiment and this one?
- B. Are the concentrations and volumes right? (Are concentrations listed those of the stocks, or are they the "final concentrations" once everything has been mixed together?)
- C. If you're using a kit, are you completely following the kit's directions? (Example: the malachite green kit for Pi detection requires the use of buffers and reagents not exposed to Pi-containing glassware etc. And it doesn't work if you add more of Reagents A and B than recommended!) Are you mixing components as thoroughly as needed? Are your incubation times adequate? Have you added reagents in the right sequence?
- D. Are you in the linear range of your detection modality? (Should enough product be present in the sample to distinguish it from a negative control? Conversely, might your signal be saturating your instrument? Beware of absorbances above 1.0!)

III. Next steps

- A. Are you not sure exactly what you just did? If so, repeat the assay more carefully, taking detailed notes.
- B. If studying a multistep reaction (e.g., by using coupling enzymes to detect a product), consider breaking the reaction down into individual steps to see which part is problematic. Example: in pathway $A \Rightarrow B \Rightarrow C \Rightarrow D$, first start with compound C and see if you can produce and detect D. If that works, then start with B and see if you can still get to D....

C. Did the assay "just barely" work before? If so, the fact that it's not working now is unsurprising. Can the protocol be improved? Or should it be abandoned altogether?

D. If you can't decide exactly what went wrong, try to identify the most likely culprit and design a simple experiment with appropriate controls to determine whether your suspicions are correct.

E. If you are having trouble detecting a product of a reaction, do you have the option of detecting another product, or detecting consumption of a substrate?