**Development of enzyme activity assays for high-throughput screening (HTS)**
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1. Is the enzyme of interest because of its likely importance in the pathogen (metabolic role? genetic/chemical validation? expression data?), druggability, uniqueness relative to human enzymes, etc.?

2. What is the enzyme's assayability? That is, are its substrates readily obtainable at a reasonable cost? Does it consume a HTS-detectable substrate, and/or can it produce a HTS-detectable product (either directly or with coupling enzymes)? Review the literature to see what others have tried, either for the enzyme you're studying or for isoforms from other species. Find and/or order reagents, including buffer and product (for making standard curves).

3. Is the enzyme active? You can test this with a "low-throughput" instrument such as a UV/visible spectrophotometer, if convenient. Do a control with a "random" enzyme to make sure that the activity is due to the recombinantly expressed enzyme of interest rather than a contaminant from the expression system. Also do controls with one substrate but not the other, both substrates but no enzyme, etc. What is the enzyme's specific activity at saturating substrate concentrations? How does this compare to literature values?

4. What are the enzyme's approximate $K_m$'s for its substrates? You can get these numbers from "low-throughput" assays, if convenient. How do they compare to literature values? $K_m$ estimates allow you to set HTS assay concentrations can be set to ~3X each $K_m$, assuming that this doesn't interfere with the detection strategy. When estimating $K_m$'s, make sure that [substrate] is well in excess of [enzyme].

5. Try the assay in HTS format (96- or 384-well plate). Some questions to consider:
- What buffer conditions maximize enzyme activity and stability? Consider variations in [salt], [glycerol], [detergent], [BSA], pH, etc.
- Is the reaction rate roughly proportional to enzyme concentration? When testing for possible inhibition of the enzyme, 50% inhibition (for example) is equivalent to reducing the enzyme concentration by 50%. Thus it's important to see that specific reductions in enzyme concentration lead to equivalent reductions in reaction rate.
- How stable are the enzyme, other reagents, and readout of the enzyme's activity? Must enzyme stocks be used as soon as they are thawed? Must any reagents be made fresh each day? Must plates be read right away?
- How does the enzyme perform when stored in 50% glycerol (to avoid freeze/thaw cycles)?
- Will the screen be performed in "kinetic" or "end-point" mode, and how long will the reaction incubation last? Enzyme activity should stay relatively constant throughout this period.
- What known (control) inhibitors can be used for assay validation? What are their $IC_{50}$s?
- What Z-factors can be achieved in comparing wells with and without enzyme, or with and without inhibitor? These should regularly exceed 0.5.

References:

T. D. Y. Chung and D. J. Murphy. Enzyme screens. In: R. Seethala and P. B. Fernandes (eds.), *Handbook of Drug Screening*, 2001.

J. H. Zhang, T. D. Chung, and K. R. Oldenburg. A Simple Statistical Parameter for Use in Evaluation and Validation of High Throughput Screening Assays. *J. Biomol. Screen.* **4**:67–73, 1999.