Zymosense Enzyme Activity Sensors

Zymosense has developed an enzyme activity assay that uses fluorescent nanoparticles noncovalently suspended in enzyme substrates to produce ready-to-use activity probes. This platform works with a variety of different enzyme substrates which feature both hydrophilic and hydrophobic moieties.

Zymosense's assay utilizes the high sensitivity of nanoparticles to their immediate surroundings to track enzyme substrate modification in real time. These particles quench in response to activity and generate responses which can then be fitted to simple kinetic models.

Due to the direct manner of substrate interrogation, this method has a broad dynamic range and relatively straightforward procedure: the probes are mixed with their target buffer and allowed to equilibrate; then, a fluorescent signal baseline is collected; next, enzyme is added to the fluorescent probes; and, lastly, fluorescent signal is collected. Fluorescence can be measured as an endpoint or as a kinetic scan.

Protease Assay

The Zymosense protease assay utilizes protein-wrapped nanotubes to track proteolytic degradation of a target substrate with potential for real-time resolution. This method typically uses albumin as a substrate for its broad pH range of solubility; however, this method can be used with a variety of soluble protein substrates.

This method exhibits a dynamic range that spans three orders of magnitude and employs a simple mix-and-measure procedure.

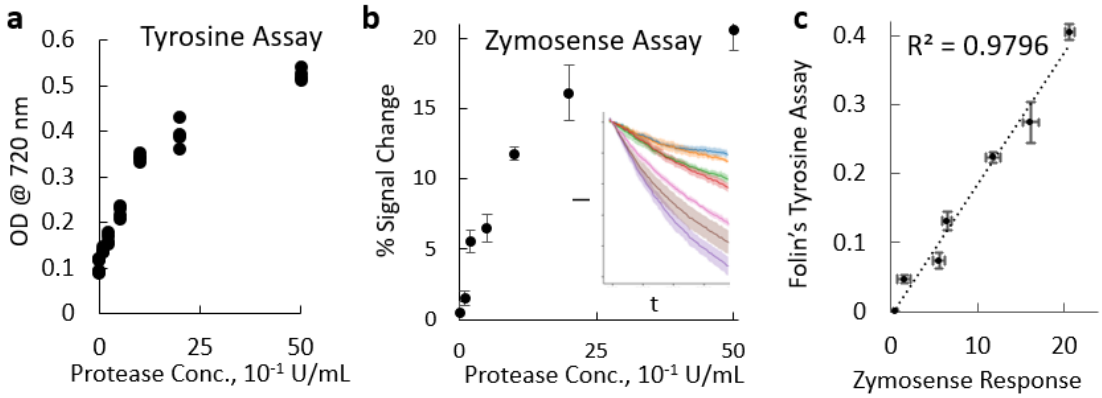

Figure 1. Comparison of tyrosine release assay (using Folin-Ciocalteu reagent as indicator) (a) with Zymosense assay (b) to determine parity (c).

<u>Complicating Factors</u>:

- Presence of organic solvents: organic solvents sometimes used for solubilizing hydrophobic enzymes can cause precipitation of protein substrates. Special substrates may be needed for these kinds of enzymes.
- High background protein concentrations: Because proteases will interact with most proteins, presence of additional proteins will change the apparent enzyme kinetics. The overall concentration of protein must be considered if attempting to collect absolute kinetic measurements. Excessively high background protein concentrations can also compete with activity probes and diminish the intensity of sensor responses.

Cuvette procedure

1) Dilute sensors x into assay buffer.
2) Add 900 µL of diluted sensor solution into cuvette.
3) Put cuvette into reader and initiate scan.
    a. For manual reading with Zymosense portable reader:
        i. Plug non-branched end of USB splitter into battery.
        ii. Press down tab on fluorimeter screen panel to initialize system and begin scanning.
        iii. Slide ring over laser pen to hold down ON-button.
    b. For data logging:
        i. Connect USB-B port of microcontroller to computer via USB cable.
        ii. Plug other components into portable battery via USB splitter.
        iii. Type desired filename into Python prompt.
        iv. Slide ring over laser pen to hold down ON-button.
        v. Initiate scan via Python code.
4) Collect signal baseline for 3-5 minutes to confirm signal stability.
5) Add 100 µL of enzyme (or control buffer) solution to the cuvette.
6) Log initial fluorescence intensity immediately after sample addition.
7) Let incubate for 30min*.
8) Collect final fluorescence intensity measurement immediately after incubation.
9) Record change in signal as enzyme activity measurement. If collecting data in real-time, plot data points to ensure enzyme-limited kinetics (straight line response).

*If concentration of enzymes are high (>30% signal change)/low (<1% signal change) incubation time can be decreased/increased to adjust the dynamic range.

96-well Plate Procedure:

1) Dilute sensors _x into assay buffer.

2) Plate 80 µL of the diluted sensor solution into well-plate* wells.
3) Load enzymes and/or controls into secondary well-plate**
4) Home excitation source on center of empty well*** using Zaber motion control program and then move the wellplate in 9mm increments until at the well to the left of the starting well.
5) Initiate scan:
    a. If performing kinetic scan:
        i. Initiate scan and collect baseline fluorescent signal for 3-5 minutes.
            1. To initiate scan, open the well-plate reader script and input the below information:
                a. Total scan time
                b. Time of sample addition (if collecting a baseline signal first)
                c. Output file name
                d. First and last wells to scan (top left and bottom right)
        ii. Ensure that photodetector and excitation source are ON, then execute scanning script.
        iii. When scan pauses, add enzyme samples to sensor wells and continue scan.
        iv. Record activity as either fitted kinetic parameter or endpoint signal change.
    b. If performing endpoint scan:
        i. Add enzyme to wells and immediately perform initial scan, inputting below information:
            1. Total scan time (set to 1 second)
            2. Time of sample addition (if collecting a baseline signal first)
            3. Output file name
            4. First and last wells to scan (top left and bottom right)
        ii. Ensure that photodetector and excitation source are ON, then execute scanning script.
        iii. Let incubate for 30 minutes.
        iv. Perform second scan for endpoint measurements.
        v. Record activity as initial-end change in signal.

*Cell culture-treated plates generally work best for achieving stable backgrounds, as they minimize non-specific protein binding to the walls of the well.
**Round-bottom well plates work the best for easily aspirating samples.

***Using an empty well minimizes any temperature biases from over-exposing any samples.