

Flow of an Enzyme Characterization (Activity Profile) Experimental Pathway

Begin with SOME amount of enzyme preparation (known volume and tissue protein concentration). Incubate with a known concentration of substrate at a pH and temperature that you think reflects the pH and temperature of the whole cell/tissue (natural conditions). Monitor product formation. Adjust concentrations of enzyme preparation and substrate so that product formation occurs in a reasonable time (less than 5 minutes for class, less than 1 hour for profession). This becomes the baseline assay system.

Parameters that are typically examined when characterizing an enzyme activity: These tests can be performed on crude preparations (simple homogenates), OR on purified material.

1. Monitor enzyme activity with varying **substrate concentration**. Maintain enzyme at the same concentration and test 5 different substrate levels, at, above and below the baseline amount.
2. Monitor enzyme activity with **varying pH**. Maintain enzyme and substrate at concentrations giving a reasonable reaction time, then use the baseline pH plus several conditions that are more acidic and more basic.
3. Monitor enzyme activity with **varying temperature**. Use the baseline temperature plus several temperatures in intervals of 10C above and below this standard.
4. Monitor enzyme activity in the presence of **various salts, soaps, lipids, metals, chelators, analogues** (compounds with similar structure/shape/chemistry) of the substrate.
5. Monitor enzyme activity on a per total protein basis in varying cell fractions.
6. Examine enzyme activity in different physiological or developmental phases (growing, resting).
7. How is the enzyme distributed in different tissues/organs (plant seeds, leaves, stems, roots, skin, pulp, etc)?
8. How do enzyme profiles compare among species? Yeast, mushrooms, potatoes, apples, bananas, lettuce, etc.