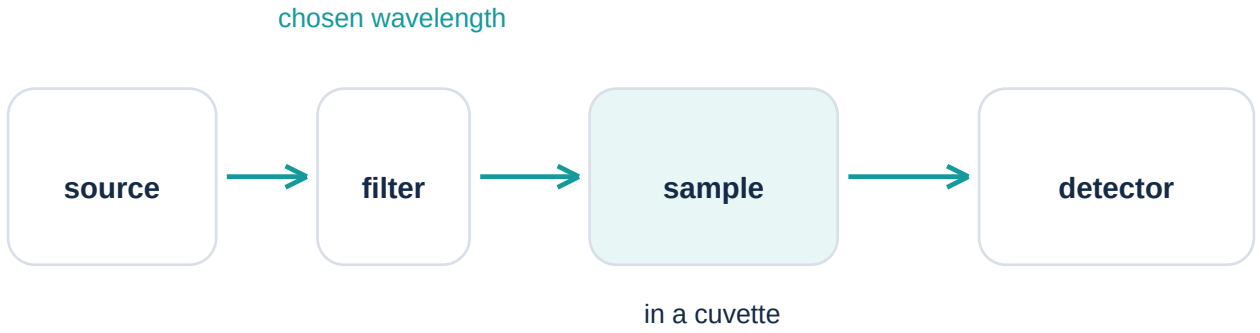
Turn colour into a number

A colorimeter sends light of a selected wavelength through a sample. Measuring transmitted light gives an objective signal that can follow a colour-changing enzyme reaction over time.

THE LIGHT PATH



Some light is absorbed by the coloured substance. The detector measures the light that passes through; the instrument reports transmittance or calculates absorbance relative to a blank.

1. ABSORBANCE AND TRANSMITTANCE MOVE OPPOSITELY

At a fixed wavelength, more absorption means higher absorbance (A) and lower transmittance (%T). A paler sample of the same coloured substance usually gives lower A and higher %T when all other conditions are unchanged.

2. MATCH THE LIGHT TO THE COLOURED SUBSTANCE

Use the method's recommended filter or wavelength, where the substance absorbs strongly. Its visible colour is mainly the light it transmits, not the light it absorbs. Keep the wavelength fixed throughout a comparison.

Absorbance is dimensionless, not a percentage. Transmittance is often displayed as a percentage. Neither reading is automatically a concentration or a reaction rate.

Instrument reference: Vernier Colorimeter user manual.

Scope: Cambridge Biology 9700 syllabus, section 3.1, outcome 4, p. 20.

Collect a reliable time series

Decide which coloured substance the signal represents. Some reactions can be read directly in a cuvette; others require timed samples mixed with a colour reagent.

1. SET UP AND ZERO WITH A BLANK

Select the wavelength and let the instrument warm up as instructed. Use a method-appropriate blank that accounts for solvent and reagent background but excludes the measured colour signal. Zero absorbance (equivalent to 100% transmittance).

2. START THE REACTION AND RECORD TIME

Mix enzyme and substrate consistently and start timing immediately. Keep temperature, pH, concentrations and volumes constant except the factor being investigated. Take readings at regular intervals; record actual times and minimise the delay to the first reading.

3. EXAMPLE: AMYLASE AND THE STARCH-IODINE SIGNAL

Withdraw equal samples into separate, fixed amounts of iodine reagent using a validated method. Standardise dilution and the delay before reading. As starch is broken down, the blue-black signal weakens and absorbance falls. Do not return tested samples to the reaction.

4. KEEP THE OPTICAL CONDITIONS CONSISTENT

Use the same light-path length and cuvette orientation, with clear faces across the beam. Wipe off fingerprints and remove bubbles; fill above the beam. Close the instrument lid. Use suitable blanks and avoid cloudy samples that scatter light.

Blank is not the same as a no-enzyme control. The blank sets the optical reference; a separate no-active-enzyme control tests whether the signal changes without enzyme catalysis. Repeat runs to judge reliability.

Method background: Idaho State University, spectrophotometers.
Starch method: Practical Biology, starch concentration calibration.

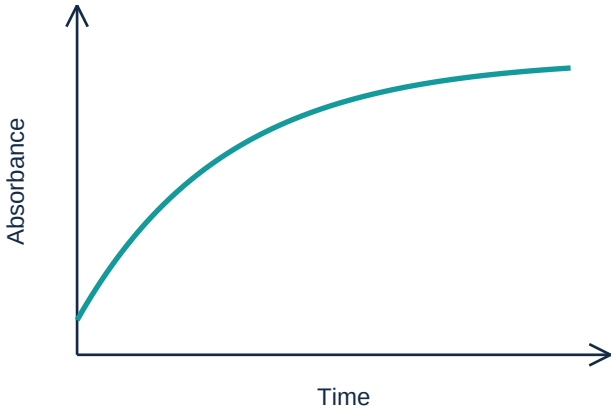
Scope: Cambridge Biology 9700 syllabus, section 3.1, outcome 4, p. 20.

Connect the signal to the reaction

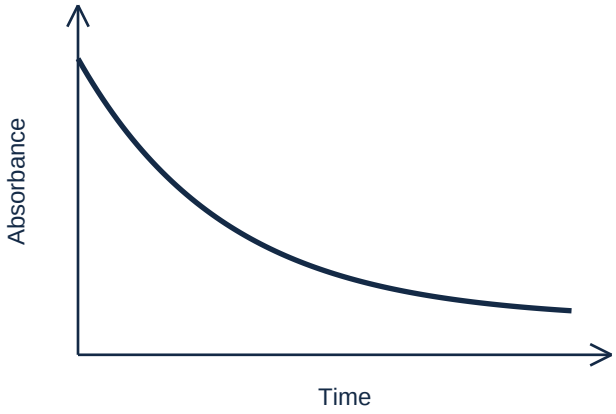
Always identify what absorbs the chosen light. Product formation can increase absorbance; loss of a coloured substrate or substrate-linked signal can decrease it.

TWO POSSIBLE PROGRESS CURVES

Coloured product forms



Substrate-linked colour fades



1. RATE DEPENDS ON CHANGE, NOT ONE READING

For a comparable assay, use change in absorbance / change in time. Illustrative fall: from 0.80 to 0.50 in 30 s gives a slope of -0.010 absorbance units s^{-1}. Report the magnitude of the decrease as 0.010 absorbance units s^{-1}. A high reading alone does not mean a fast reaction.

2. EXTENSION: CONVERT THE SIGNAL TO CONCENTRATION

Measure standards of known concentration under the same conditions and plot absorbance against concentration. Use this calibration curve to convert sample readings within its valid range. Apply any dilution correction before calculating a concentration change per time.

For an initial rate, use the initial tangent or a justified early linear interval. Compare slopes only with matching assay conditions. A flattening curve shows less signal change, not proof that all enzymes have denatured.

Rate interpretation: DePaul University, Enzyme Assays.
Calibration: Practical Biology, starch concentration calibration.

Scope: Cambridge Biology 9700 syllabus, section 3.1, outcome 4, p. 20.