

Product Information

Amylase Nanoassay™

Catalog Number: AMYSTRNNP

Product Description: Amylase Nanosensor™
Assay Kit

Starch particles that are designed to report amylolytic activity via a decrease in light scattering.

Materials:

- 1) Nanosensor Solution – 1 bottle
- 2) Reaction Buffer – 2 bottles pH 7 HEPES buffer

Content	1 mL	Target Enzyme	Amylase
Shipping Temp	Ambient	Assay format	Absorbance spectrometer; Well plate
Storage Temp	4° C	Detection method	Absorbance
Physical form	Suspension	Wavelength(nm)	450 (adjustable)
Stability	>1 year		

Nanoassay™ Overview:

This approach uses engineered nanoparticles made of starch to monitor amylolytic activity in real time via light scattering. As the amylose is hydrolyzed, the molecule is solubilized, and the solution is clarified. A decrease in light attenuation, as measured by absorbance, indicates hydrolytic activity.

Amylose Nanoparticle Light Scattering Assay for Amylase Activity

To measure amylase activity using an amylose nanoparticle light scattering assay:

1. **Prepare assay conditions**
 - 1.1) Pre-heat the plate reader (and plate, if possible) to the desired temperature (e.g., 37 °C).
 - 1.2) Set reader to kinetic absorbance (turbidity/light scattering) mode at your established wavelength.
2. **Prepare the sensor dispersion**
 - 2.1) Mix the amylose nanoparticle sensor dispersion thoroughly (invert several times; gentle vortex if allowed).
 - 2.2) Dilute the sensor dispersion 100-fold in assay buffer (e.g., 1:99 sensor:buffer).
 - 2.3) Keep diluted sensor gently mixed until loading to minimize settling.
3. **Prepare controls and standards**
 - 3.1) Prepare a sensor-only negative control (diluted sensor + buffer; no enzyme).
 - 3.2) (Optional) Prepare an amylase concentration/activity series for a relative standard curve.
 - 3.3) (Optional; recommended for dark/turbid samples) Prepare a **sample-only control** for each sample type (sample + buffer; **no sensor**) to quantify matrix background and drift.
4. **Load the plate**
 - 4.1) Add 100 µL of the 100×-diluted sensor to each sensor well.
 - 4.2) Allocate wells for:
 - Sensor-only negative control (replicates)
 - Sensor + enzyme standards (if used)
 - Sensor + unknown samples (replicates)
 - (Optional) Sample-only controls (sample + buffer; no sensor; replicates)
5. **Seal and equilibrate (optional)**
 - 5.1) Cover the plate with a plate seal/film (recommended to reduce evaporation and splashing during mixing).
 - 5.2) Allow plate to equilibrate at temperature for ~2–5 minutes (optional but helps baseline stability).
6. **Program the kinetic method**
 - 6.1) Measurement: **Absorbance** at your chosen wavelength (450nm used in clear samples) (scattering/turbidity tracking).
 - 6.2) Baseline segment: collect a short baseline (e.g., **1–5 minutes**) prior to enzyme addition.
 - 6.3) Mixing between reads (to minimize settling):
 - Orbital mixing: **~400 rpm**
 - Orbit: **2 mm radius**
 - Duration: **5 seconds**
 - Apply **between each plate scan**
 - 6.4) Post-addition kinetic duration: **10–60 minutes**.
7. **Collect baseline**
 - 7.1) Start the kinetic run and collect baseline reads (no enzyme present).
8. **Add enzyme and resume kinetic scan**
 - 8.1) Pause the run (or eject plate if required).
 - 8.2) Add enzyme (or amylase-containing sample) to the appropriate wells using consistent

timing and volume.

8.3) For negative controls:

- Sensor-only negative control: add buffer/vehicle instead of enzyme (same volume).
- **Sample-only controls (if used):** add the same enzyme volume to the sample-only wells if you are trying to subtract absorbance of the sample

8.4) Re-cover the plate promptly and resume scanning immediately.

8.5) Continue kinetic reads for the planned duration with mixing between each scan.

To analyze data:

1. Review data quality

- 1.1) Flag wells with bubbles, splashing under the seal, or obvious pipetting errors.
- 1.2) Confirm sensor-only negative controls are stable (minimal drift over time).

2. (Optional) Apply smoothing

- 2.1) Apply a rolling average (e.g., 5 points) if traces are noisy.

3. (Optional; for dark/turbid samples) Background-correct using sample-only controls

3.1) For each sample type, compute corrected absorbance:

$$\circ A_{\text{corr}}(t) = A_{\text{sensor+sample}}(t) - A_{\text{sample-only}}(t)$$

3.2) Use $A_{\text{corr}}(t)$ for all remaining steps.

3.3) If you did not run sample-only controls, proceed using raw $A(t)$.

4. Normalize absorbance at the end of the scan (endpoint normalization)

4.1) Choose the endpoint/time window (e.g., last 1–3 minutes).

4.2) For each well, determine the final absorbance value A_{final} (or $A_{\text{corr,final}}$).

4.3) Normalize the full trace:

$$\circ A_{\text{norm}}(t) = A(t)/A_{\text{final}} \text{ (or } A_{\text{corr}}(t)/A_{\text{corr,final}})$$

5. Calculate percent change in absorbance (relative activity)

5.1) Define baseline A_{base} as the average of baseline points either immediately before enzyme addition or immediately after – depending on the optical activity of the enzyme sample and/or severity of initial burst phase kinetics.

5.2) Compute:

$$\circ \% \Delta A = 100 \times \left(\frac{A_{\text{norm,final}} - A_{\text{norm,base}}}{A_{\text{norm,base}}} \right)$$

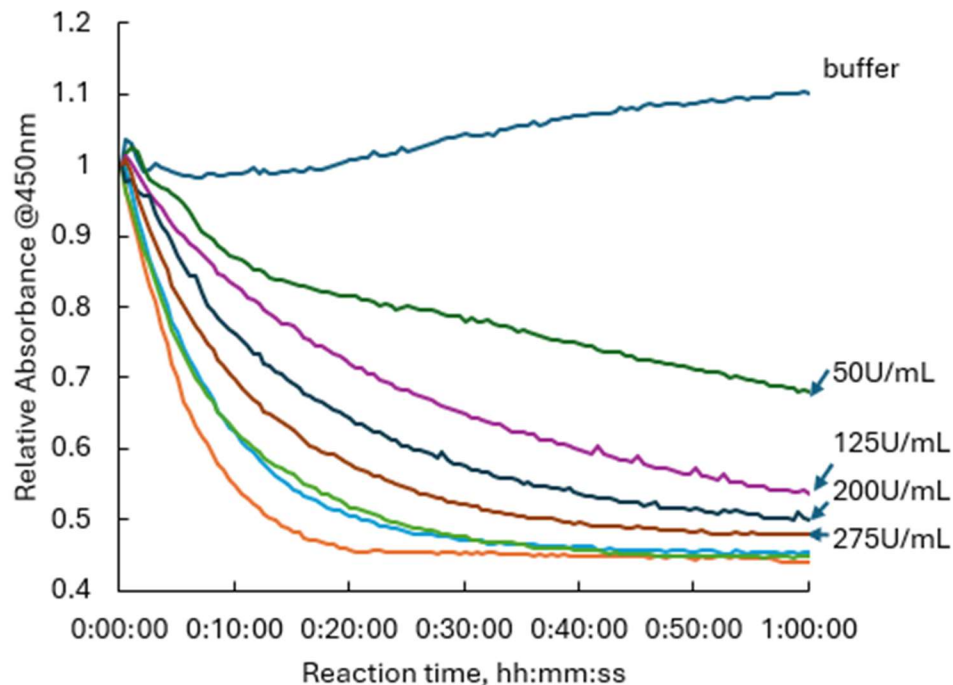
(Because endpoint normalization makes $A_{\text{norm,final}} \approx 1$, this is effectively a baseline-relative % change anchored to the endpoint.)

6. Report results

6.1) Report $\% \Delta A$ (mean \pm SD across replicates).

6.2) If using a standard curve, map $\% \Delta A$ to relative activity units for comparing runs/days.

Sample Kinetic Scan of Amylase



Troubleshooting:

1. Highly noisy erratic signal response

Large particulates are present – either from precipitation of the sensor or from the sample. Dilute enzyme samples further to reduce matrix effects.

2. Little/no absorbance of sample

Sensor nanoparticles solubilized. Solubilization can occur from hydrolysis over time or heat. Run assay at a lower temperature, different buffer, or seek replacement assay.

3. Large absorbance shift immediately following enzyme addition.

a. Optical density goes up

Can be caused by highly opaque sample. Can be resolved either by dilution or use of an additional sample blank (enzyme sample + water).

b. Optical density goes down

Burst phase enzyme kinetics may have depleted enzyme substrate. Try further diluting the enzyme sample to be within dynamic range.

4. Large background increase to absorbance over time

If controls are gradually increasing in optical density, it is a sign that sedimentation is occurring. Try increasing orbital mixing frequency and/or shortening orbital radius.

Example Standard Curve:

