ZYMOSENSE
The Nanoassay Company

sales@zymosense.com
2501 North Loop Drive, Suite 1010, Ames, IA 50010 (515) 515-7299

Product Information

Phytase Nanoassay™

Catalog Number: PHYPHYNNP

Product Description: Phytase Nanosensor™ Assay Kit

Phytate-containing particles that are designed to report phytase activity via a decrease in light scattering.

Materials:

1) Nanosensor Solution – 4 bottles
2) Reaction Buffer – 2 bottles pH 5.5 MES buffer

Content	1 mL	**Target Enzyme**	Phytase
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 containing phytate to monitor phytase activity in real time via light scattering. As the phytate is hydrolyzed, the particle is solubilized, and the solution is clarified. A decrease in light attenuation, as measured by absorbance, indicates hydrolytic activity.

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Phytate Nanoparticle Light Scattering Assay for Phytase Activity
To measure phytase activity using an phytate nanoparticle light scattering assay:

1. **Prepare assay conditions**
   1.1) Set plate reader to room temperature (22-25° 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 phytate nanoparticle sensor dispersion thoroughly (invert several times; gentle vortex if allowed).
3. **Prepare controls and standards**
   3.1) Prepare a sensor-only negative control (diluted sensor + buffer; no enzyme).
   3.2) (Optional) Prepare a phytase 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 for long kinetic scans).
   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 phytase-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

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

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

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

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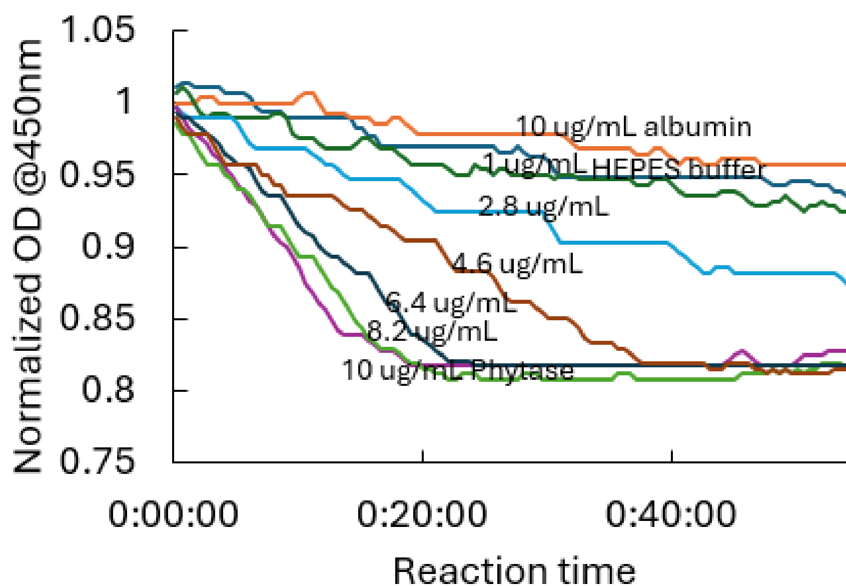
Sample Kinetic Scan of Phytase



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.

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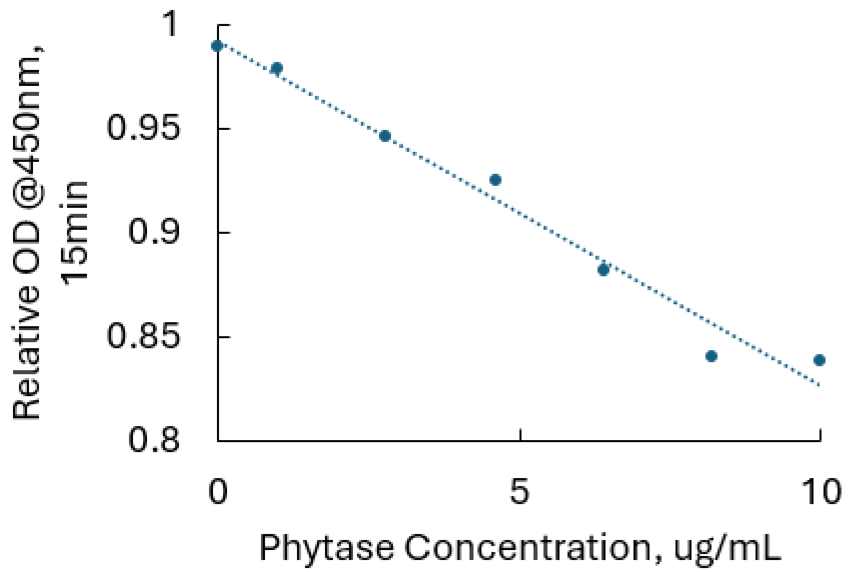
Example Standard Curve:



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