

EnzChek® Ultra Phytase Assay Kit

Catalog no. E33701

Table 1. Contents and storage information.

Material	Amount	Storage	Stability
Amplex® UltraRed reagent, MW = ~300 (Component A)	5 vials, each containing 0.18 mg	<ul style="list-style-type: none"> • ≤-20°C • Desiccate • Protect from light 	When stored as directed this kit is stable for 1 year.
Dimethylsulfoxide (DMSO), anhydrous (Component B)	500 µL		
10X Reaction Buffer (Component C)	28 mL of 1 M sodium acetate, pH 5.5		
Phytic acid, dodecasodium salt hydrate, FW = 923.8 (Component D)	65 mg, 85% by weight		
Maltose phosphorylase, recombinant from <i>Escherichia coli</i> (Component E)	150 U*		
Glucose oxidase, from <i>Aspergillus niger</i> (Component F)	200 U†		
Horseradish peroxidase (HRP) (Component G)	30 U‡		
Maltose, monohydrate, MW = 360.3 (Component H)	20 mg		
Phosphate standard (Component I)	500 µL of 50 mM potassium phosphate		

Number of assays: Each kit contains sufficient reagents for five 96-well plates at 6 mL per plate based on the protocol below, with ~20% excess for pipetting considerations or for partial-plate assays.

Approximate fluorescence excitation/emission maxima: Amplex® UltraRed reagent: ~568/581 nm.

* 1 unit = the amount of enzyme that will convert maltose, in the presence of inorganic phosphate, to 1.0 µmole of D-glucose and D-glucose 1-phosphate per minute at 37°C, pH 7.0.

† 1 unit = the amount of enzyme that will oxidize 1.0 µmole of β-D-glucose to D-gluconolactone and H₂O₂ per minute at 35°C, pH 5.1.

‡ 1 unit = the amount of enzyme that will form 1.0 mg purpurogallin from pyrogallol in 20 seconds at 20°C, pH 6.0.

Introduction

Phytases catalyze the sequential hydrolysis of phytate (myoinositol hexakis phosphate; phytin; phytic acid) to less phosphorylated myo-inositol compounds and inorganic phosphate. This kit detects phytase activity based on measurement of the phosphate released from the substrate phytic acid.

The EnzChek® Ultra Phytase Assay Kit utilizes a series of linked enzymatic reactions for the detection of phytase activity (Figure 1). In the initial step, phytase catalyzes the release of inorganic phosphate from phytic acid; in the presence of P_i , maltose phosphorylase converts maltose to glucose 1-phosphate and glucose. Glucose oxidase then converts the glucose to gluconolactone and H_2O_2 . Finally, with horseradish peroxidase (HRP) as a catalyst, the H_2O_2 reacts with the Amplex® UltraRed reagent to generate a fluorescent product, which has absorption and fluorescent emission maxima of ~568 nm and 581 nm, respectively (Figure 2). The resulting increase in fluorescence is proportional to the amount of P_i in the sample. This relative measure of phytase activity can be used as the basis of a standard curve of phytase enzyme standards with known activity.

The assay takes one hour at 37° C; under these conditions, the limit of detection is 0.001 FTU/mL and the intra-assay CV is $\leq 10\%$. The signal is not linear but can cover a two-log concentration range for phytase (Figure 3).

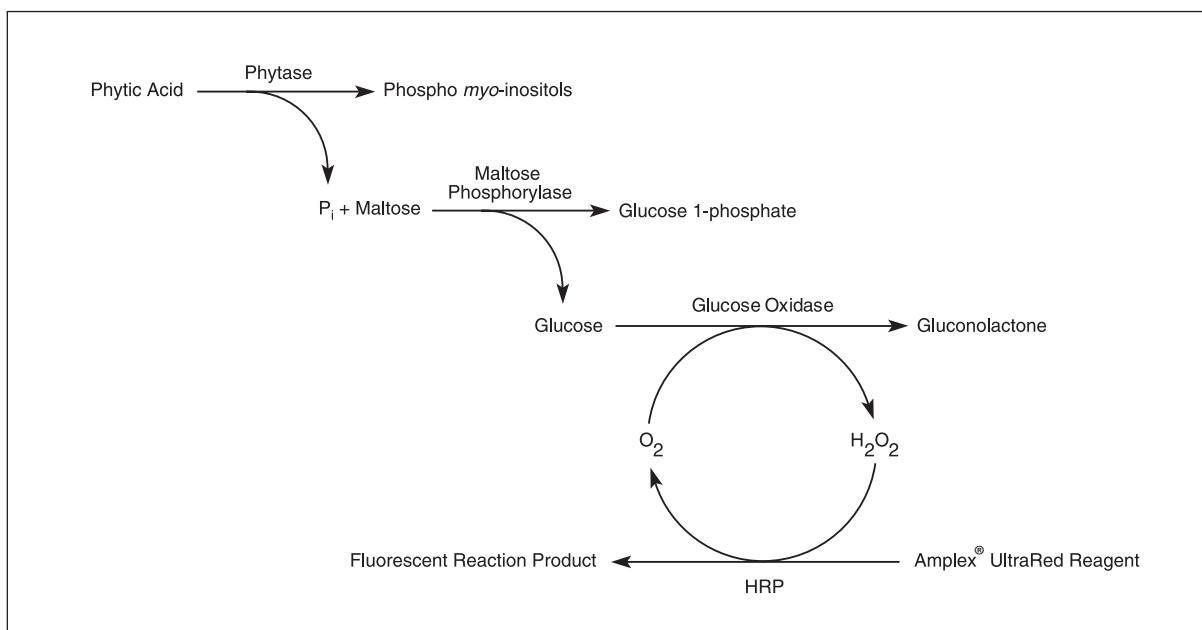


Figure 1. Principle of EnzChek® phytase assay.

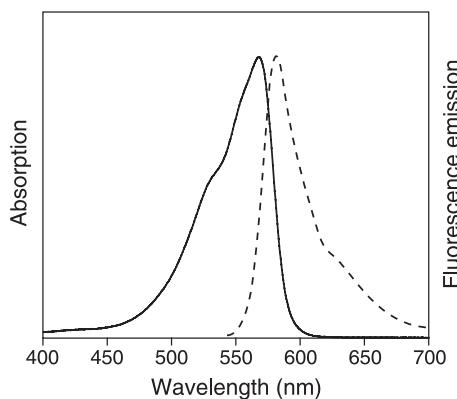


Figure 2. Normalized absorption and fluorescence emission spectra for the Amplex® UltraRed peroxidation product.

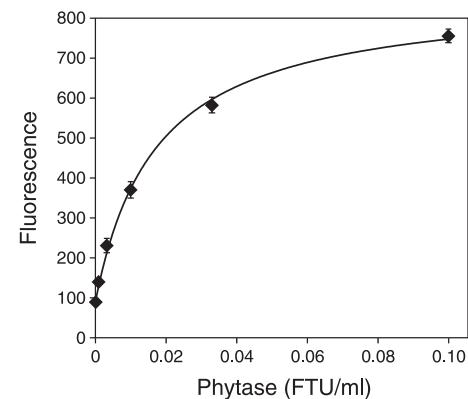


Figure 3. Assay of a standard phytase preparation using the EnzChek® Ultra phytase assay. A dilution series of Natuphos® 10000 L phytase (BASF) in 0.1 M acetate buffer (pH 5.5) was assayed with the EnzChek® Ultra Phytase Assay Kit. Incubation time was 60 minutes; phytase activity is shown in FTU/mL (FTU= phytase unit, as defined by the manufacturer). The data can be fit to a hyperbolic equation.

Before Starting

Materials Required But Not Provided

- Deionized water (dH₂O)

Caution

DMSO is known to facilitate the entry of organic molecules into tissues. Handle reagents containing DMSO (e.g., Amplex[®] UltraRed reagent stock solution in DMSO) using equipment and practices appropriate for the hazards posed by such materials. Dispose off the reagents in compliance with all pertaining local regulations.

Preparing Solutions

10 mM Amplex[®] UltraRed reagent stock solution

- 1.1 To prepare a 10 mM stock solution of Amplex[®] UltraRed reagent, allow one vial of Amplex[®] UltraRed reagent (Component A) and the DMSO (Component B) to warm to room temperature. Immediately prior to use, dissolve the contents of the vial of Amplex[®] UltraRed reagent in 60 µL DMSO. Each vial of Amplex[®] UltraRed reagent is sufficient for approximately 120 assays, with a final reaction volume of 100 µL per assay. Store the stock solution frozen at ≤-20°C, protected from light.

1X Reaction Buffer working solution

- 1.2 To prepare a 1X working solution of Reaction Buffer (0.1 M sodium acetate, pH 5.5), add 2.5 mL of 10X Reaction Buffer stock solution (Component C) to 22.5 mL of deionized water (dH₂O). This 25 mL volume of 1X Reaction Buffer is sufficient to formulate the stock solutions and dilute the reaction buffer, enzymes, and standards.

40 mM Phytic Acid

- 1.3 To prepare a 40 mM solution of phytic acid, dissolve the contents of the vial of phytic acid (Component D) in 1.5 mL of 1X Reaction Buffer. Store any remaining phytic acid solution frozen at ≤-20°C.

200 U/mL maltose phosphorylase stock solution

- 1.4 To prepare a 200 U/mL stock solution of maltose phosphorylase, dissolve the contents of the vial of maltose phosphorylase (Component E) in 750 µL of 1X Reaction Buffer. Store any remaining maltose phosphorylase solution frozen at ≤-20°C.

200 U/mL glucose oxidase stock solution

- 1.5 To prepare a 200 U/mL solution of glucose oxidase, dissolve the contents of the vial of glucose oxidase (Component F) in 1.0 mL of 1X Reaction Buffer. Store any remaining glucose oxidase solution frozen at ≤-20°C.

100 U/mL horseradish peroxidase (HRP) stock solution

- 1.6 To prepare a 100 U/mL stock solution of horseradish peroxidase (HRP), dissolve the contents of the vial of HRP (Component G) in 300 µL of 1X Reaction Buffer. Store any remaining HRP solution frozen at ≤-20°C.

40 mM maltose stock solution

- 1.7 To prepare a 40 mM stock solution of maltose, dissolve the contents of the vial of maltose (Component H) in 1.39 mL of 1X Reaction Buffer. Store any remaining maltose solution frozen at ≤-20°C.

Experimental Protocols

General Considerations

The dependence of this assay on phytic acid as a phosphate source is diagnostic for phytase activity in an unknown mixture. The phytic acid substrate provided in this kit is of the highest quality commonly available; nevertheless, at 1 mM phytic acid, we observe 10–20 µM phosphate-equivalent background.

The EnzChek® phytase assay utilizes a linked-enzyme system for the accurate detection of free phosphate. Contaminants that alter the activity of the enzymes in this assay system can interfere with correct phytase activity determination; common interfering agents may include phosphate, glucose, and reducing agents such as DTT. In the presence of a high excess of phosphate, the Amplex® UltraRed reagent can be further oxidized to a nonfluorescent state, resulting in a decrease in the observed signal and an inaccurate assessment of phytase activity.

Phytase Activity Assay

- 2.1 Prepare a standard curve by diluting a sample of known phytase activity in 1X Reaction Buffer (prepared in step 1.2) to a final range of 0.001 to 0.1 FTU/mL, with 1X buffer as a negative (no enzyme) control. A volume of 50 µL will be used for each reaction.
- 2.2 Prepare a dilution series of the unknown phytase sample sufficient to span the activity range covered by the standard curve.
- 2.3 If desired, prepare a positive control for phosphate detection by diluting the 50 mM phosphate standard (Component I) to 1–100 µM (20 µM works well) in 1X Reaction Buffer. The effective phosphate concentration range for this kit is 1–200 µM.
- 2.4 Pipet 50 µL of the diluted samples and controls into separate wells of a microplate.
- 2.5 Prepare a working reaction mixture of 100 µM Amplex® UltraRed reagent containing 4 U/mL maltose phosphorylase, 0.4 mM maltose, 2 U/mL glucose oxidase, 0.4 U/mL HRP and 2 mM phytic acid.

Note: A 96-well plate generally requires at least 10% excess (5.5 mL). To prepare 3, 4, 5, or 6 mL of this reaction mixture (as needed), refer to Table 1. Prepare only the amount needed for the experiment at hand; use the reaction mixture within 5 minutes of preparation.

Table 2. Guide to preparing the reaction mixture.

Volume of Reaction Mixture Needed	Use						
	1X Reaction Buffer	40 mM Phytic Acid	10 mM Amplex® UltraRed Reagent	Maltose Phosphorylase	Glucose Oxidase	Horseradish Peroxidase	Maltose
3 mL	2.7 mL	150 µL	30 µL	60 µL	30 µL	12 µL	30 µL
4 mL	3.6 mL	200 µL	40 µL	80 µL	40 µL	16 µL	40 µL
5 mL	4.5 mL	250 µL	50 µL	100 µL	50 µL	20 µL	50 µL
6 mL	5.4 mL	300 µL	60 µL	120 µL	60 µL	24 µL	60 µL

- 2.6 Begin the reactions by adding 50 µL of the reaction mixture to each microplate well containing the samples and controls.

2.7 Incubate the reactions for 60 minutes at 37°C, **protected from light**.

Note: Since the assay reaction is continuous (not terminated), you may measure the fluorescence at multiple time points to determine the rate of fluorescence production, if desired.

2.8 Measure sample fluorescence with a fluorescence microplate reader set for excitation in the range of 530–560 nm and emission detection at 580–590 nm (Figure 2).

2.9 You can obtain the phytase activity for unknown samples by using a standard curve generated from assaying samples of a phytase standard of known activity (see step 2.1). Phytase activity data generated from the EnzChek® Ultra Phytase Assay are best described by a hyperbolic curve; you can determine unknown phytase activities mathematically, using the equation for the hyperbolic curve of best fit to the standard data. Alternatively, you can evaluate unknown phytase activities by direct comparison to the phytase standard curve.

Product List Current prices may be obtained from our website or from our Customer Service Department.

Cat. no.	Product Name	Unit Size
E33701	EnzChek® Ultra Phytase Assay Kit *500 assays*	1 kit

Contact Information

Molecular Probes, Inc.
29851 Willow Creek Road
Eugene, OR 97402
Phone: (541) 465-8300
Fax: (541) 335-0504

Customer Service:
6:00 am to 4:30 pm (Pacific Time)
Phone: (541) 335-0338
Fax: (541) 335-0305
probesorder@invitrogen.com

Toll-Free Ordering for USA:
Order Phone: (800) 438-2209
Order Fax: (800) 438-0228

Technical Service:
8:00 am to 4:00 pm (Pacific Time)
Phone: (541) 335-0353
Toll-Free (800) 438-2209
Fax: (541) 335-0238
probestech@invitrogen.com

European Headquarters
3 Fountain Drive
Inchinnan Business Park
Paisley PA4 9RF, UK
Phone: +44 (0) 141 814 6100
Fax: +44 (0) 141 814 6260
Email: euroinfo@invitrogen.com
Technical Services: eurotech@invitrogen.com

For country-specific contact information,
visit www.invitrogen.com.

Further information on Molecular Probes products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Paisley, United Kingdom. All others should contact our Technical Service Department in Eugene, Oregon.

Molecular Probes products are high-quality reagents and materials intended for research purposes only. These products must be used by, or directly under the supervision of, a technically qualified individual experienced in handling potentially hazardous chemicals. Please read the Material Safety Data Sheet provided for each product; other regulatory considerations may apply.

Limited Use Label License No. 223: Labeling and Detection Technology

The manufacture, use, sale or import of this product may be subject to one or more patents or pending applications owned or licensed by Life Technologies Corporation. The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity) in a manner consistent with the accompanying product literature. The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes. The buyer may transfer information or materials made through the use of this product to a scientific collaborator, provided that such transfer is not for any Commercial Purpose, and that such collaborator agrees in writing (a) to not transfer such materials to any third party, and (b) to use such transferred materials and/or information solely for research and not for Commercial Purposes. Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research. For products that are subject to multiple limited use label licenses, the most restrictive terms apply. Life Technologies Corporation will not assert a claim against the buyer of infringement of patents that are owned or controlled by Life Technologies Corporation and/or Molecular Probes, Inc. which cover this product based upon the manufacture, use or sale of a therapeutic, clinical diagnostic, vaccine or prophylactic product developed in research by the buyer in which this product or its components was employed, provided that neither this product nor any of its components was used in the manufacture of such product. If the purchaser is not willing to accept the limitations of this limited use statement, Life Technologies is willing to accept return of the product with a full refund. For information on purchasing a license to this product for purposes other than research, contact Molecular Probes, Inc., Business Development, 29851 Willow Creek Road, Eugene, OR 97402, Tel: (541) 465-8300. Fax: (541) 335-0354.

Several Molecular Probes products and product applications are covered by U.S. and foreign patents and patents pending. All names containing the designation ® are registered with the U.S. Patent and Trademark Office.

Copyright 2010, Molecular Probes, Inc. All rights reserved. This information is subject to change without notice.

For research use only. Not intended for any animal or human therapeutic or diagnostic use.