EnzChek® Ultra *Xylanase Assay Kit (E33650)*

Quick Facts

Storage upon receipt:

- ≤–20ºC
- Protect from light

Ex/Em of reaction product: ~358/455 nm

Introduction

The hydrolysis of xylosidic linkages in hemicellulose polysaccharides by xylanase (EC 3.2.1.8) is important in a wide range of industrial processes including baking, pulp and paper manufacturing, and animal feed production. Xylanases occur in a wide variety of bacteria and fungi and are classified into two families — glycosyl hydrolase families 10 and 11.[1] Family 11 xylanases are more specific for xylans; family 10 xylanases also exhibit cellulase activity. Existing xylanase assay methods typically require separation or heating steps.[2-4] The chromogenic substrate *o*-nitrophenyl-β-D-xylobioside[5] can be used in a simpler homogeneous assay format; however, *o*-nitrophenol is pH sensitive (pK_a = 7.2) and therefore less than ideal for spectrophotometric assays of xylanases, which exhibit a wide range of pH optima (~2–9).

The EnzChek® Xylanase Assay Kit (E33650) provides the speed, sensitivity, and convenience required for measuring xylanase activity or for screening xylanase inhibitors in a high-throughput format. This kit can be used for continuous detection of xylanase activity, and offers broad dynamic and pH ranges (1.5 to 200 mU/mL and pH 4–10, respectively), high sensitivity (as low as 1.5 mU/mL), and excellent temperature tolerance.

The kit is suitable for the kinetic assay of xylanase from *Trichoderma viride*, *T. longibrachiatum*, *Thermomyces lanuginosus*, *Aspergillus niger*, and other bacterial and fungal species. Each kit contains sufficient substrate for ~500 assays in a 96-well microplate format, using a reaction volume of 100 µL per assay. The kit also contains reaction buffer and a fluorescent reference standard that can be used to determine the turnover rate of the substrate. The EnzChek Xylanase Assay Kit features a quick and convenient mix-and-read format; the fluorescence can be measured in a fluorescence microplate reader or a standard fluorometer (excitation/emission maxima ~358/455 nm).

Materials

Kit Components

- **Xylanase substrate** (Component A), five vials, each containing 250 µg
- **10X Reaction Buffer** (Component B), 10 mL of 1 M sodium acetate, pH 4.6
- **Fluorescence standard** (Component C), 212 µg

Materials required but not provided:

- Xylanase control enzyme. Control xylanase can be obtained from any commercial source as long as the unit definition is clear. For example, xylanase from *T. lanuginosus* (X2753) is available from Sigma Chemical Co., St. Louis, MO. Observed detection limit and range varies with enzyme source (Table 1).
- 96-well microplates

Each kit contains sufficient reagents for performing approximately 500 assays, using a reaction volume of 100 µL per assay.

Storage

Upon receipt, the EnzChek Xylanase Assay Kit should be stored at ≤–20°C. The xylanase substrate should be stored at ≤–20°C, desiccated and protected from light.

Stock Solution Preparation

1.1 Prepare a 1X working solution of Reaction Buffer by adding 1 mL of 10X Reaction Buffer stock solution (Component B) to 9 mL of deionized water (dH_2O). This 10 mL of 1X Reaction Buffer is sufficient for ~100 assays of 100 µL each.

Table 1. Detection limit and range for xylanases from different species.

Source	Detection Limit (mU/mL)*	Detection Range (mU/mL)*
Trichoderma viride	25	25–200
T. longibrachiatum	6.3	6.3–200
Aspergillus niger	1.5	1.5–200
Thermomyces lanuginosus	3.2	3.2–50

* Detection limit and range specifications are based on unit definitions supplied by the manufacturers.

1.2 Prepare a 1 mg/mL stock solution of xylanase substrate. Bring one vial of xylanase substrate (Component A) and the 1X reaction buffer to room temperature; add 250 µL of 1X buffer directly to the vial of substrate. This 250 µL volume is sufficient for performing ~100 assays using the protocol described below. If stored desiccated at ≤–20°C, protected from light, the xylanase substrate stock solution should remain stable for approximately one month.

1.3 Prepare a 10 U/mL stock solution of control xylanase for generating a standard curve.

1.4 If desired, prepare a 10 mM stock solution of fluorescence standard solution by adding 100 µL of dimethylformamide (DMF) directly to the vial of fluorescence standard (Component C). This solution can be used to prepare a standard curve to determine the moles of product produced in the substrate-containing reactions. This stock solution should be stored frozen at ≤–20°C, protected from light.

Experimental Protocol

The following procedure is designed for use with a fluorescence microplate reader.

2.1 Immediately prior to performing the assay, prepare a 50 µg/ mL working solution of the xylanase substrate. For example, to prepare sufficient working solution for 100 assays, add the entire amount (250 µL) of 1 mg/mL xylanase substrate stock solution (prepared in step 1.2) to 4.75 mL of 1X Reaction Buffer or the buffer of your choice (note **A**).

2.2 Dilute the xylanase-containing samples in 1X Reaction Buffer or the buffer of your choice. Pipet 50 µL of the sample preparations into microplate wells.

2.3 For the standard curve of known xylanase activity, prepare a 400 mU/mL working solution of control xylanase and dilute it 1:1 in 1X Reaction Buffer (Figure 1). Pipet 50 µL of the preparations into microplate wells. Duplicate samples are recommended.

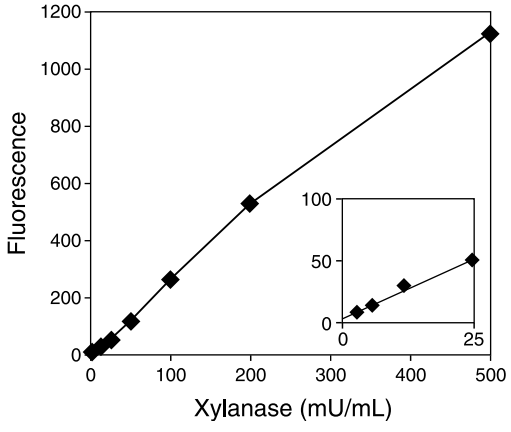

Figure 1. *Aspergillus niger* xylanase standard curve. The EnzChek xylanase assay was performed at pH 4.6 under standard conditions (30 minute incubation) as described in the experimental protocol, using *A. niger* xylanase and the EnzChek xylanase substrate. The fluorescence signal for 500 mU/mL of xylanase is equivalent to 49 µM fluorescence standard, indicating 94% turnover of the xylanase substrate (initially 52 µM).

2.4 Add 50 µL of the 50 µg/mL xylanase substrate working solution (step 2.1) into each microplate well simultaneously using a multichannel pipettor. Use 50 µL of 1X Reaction Buffer as a negative control.

2.5 Incubate the samples at room temperature, protected from light, for the desired length of time (30 minutes is recommended).

2.6 Measure the fluorescence using excitation at ~360 nm and emission detection at ~460 nm (see note **B**). The reaction product has excitation and emission maxima of ~358 nm and 455 nm, respectively. Fluorescence may be measured at multiple time points to determine the rate of product formation (step 3.2).

2.7 Determine the xylanase activity of unknown samples by equating their fluorescence intensity values with xylanase standard values (step 2.3; Figure 1) measured under identical conditions.

2.8 Prepare a fluorescent reference standard curve. Dilute the appropriate amount of 10 mM fluorescence standard stock solution (prepared in step 1.4) into 1X Reaction Buffer or the buffer of your choice (see note **A**) to yield standard solutions ranging in concentration from 0–100 µM. Pipet 100 µL of each standard into microplate wells at any time prior to measuring the fluorescence.

Calculation of Product Formation Rates from Fluorescence Intensity Data

3.1 Plot fluorescence standard concentration (µmol L^{-1}) against relative fluorescence units (RFU). The slope of this linear plot multiplied by 10^{-4} (0.0001) represents RFU/µmol of reaction product ("standard slope"). This calculation assumes that the recommended reaction volume of 100 µL (10^{-4}L) per reaction is used. Under these conditions the plot should be linear up to at least 50 µM fluorescence standard.

3.2 Measure the increase in RFU for an enzymatic reaction over a known time interval (Δt). Typically, Δt should be <5 minutes. Divide the increase in RFU by the time interval (ΔRFU/Δt)

3.3 Multiply ΔRFU/Δt by the inverse of the standard slope to obtain the product formation rate: (ΔRFU/Δt) × (µmol/RFU) = µmol/s (assuming Δt is measured in seconds) (see note **B**).

Note

[A] The reaction buffer provided can be replaced with a user-supplied buffer to match the pH optima of specific xylanases. In the pH range 4–10, no other changes to the protocol are necessary. At pH <4, the excitation/emission wavelength settings (step 2.6) should be changed to 325/460 nm. It is essential that enzyme reaction and fluorescence standard samples are prepared in the same buffer.

[B] It is essential to the validity of this calculation that the enzyme reaction and fluorescence standard measurements are carried out under identical conditions of excitation/emission wavelength, instrument sensitivity, sample volume, temperature, pH, and any other experimental parameter that influences RFU values. This condition is best satisfied by placing the enzyme reaction and fluorescence standard samples in the same microplate and making the measurements in immediate succession.

References

1. Curr Opin Biotechnol 7, 337 (1996); **2.** Meth Enzymol 160, 74 (1988); **3.** Biotechnol Lett 25, 1619 (2003); **4.** Meth Enzymol 160, 74 (1988); **5.** Biosci Biotech Biochem 60, 983 (1996).

Product List *Current prices may be obtained from our Web site or from our Customer Service Department.*

Cat #	Product Name	Unit Size
E33650	EnzChek® *Ultra* Xylanase Assay Kit *500 assays*	1 kit

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29851 Willow Creek Road, Eugene, OR 97402
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Fax: (541) 335-0238 • probestech@invitrogen.com

Invitrogen European Headquarters
Invitrogen, Ltd.
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