

EnzChek® Ultra Amylase Assay Kit (E33651)

Quick Facts

Storage upon receipt:

- Store at room temperature
- Desiccate
- Protect from light

Ex/Em maxima of digestion product:

502/512 nm

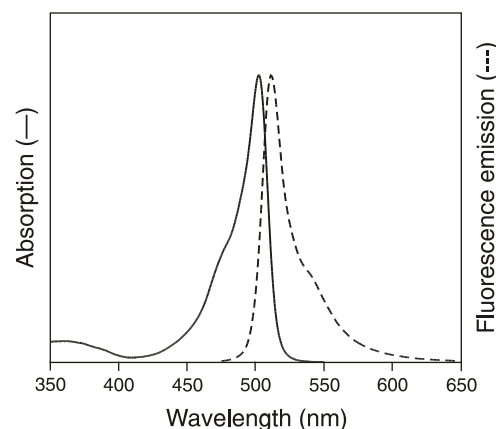


Figure 1. Normalized absorption and fluorescence emission spectra of the BODIPY FL dye.

Introduction

α -Amylase is an enzyme that catalyzes the hydrolysis of starch to a mixture of maltose, maltotriose, and dextrans. The level of α -amylase in various fluids of the human body is of clinical significance in the diagnosis of disease states including pancreatitis and diabetes; plant and microbial α -amylases are important enzymes for industrial applications ranging from the manufacture of baked goods and dairy products to the production of ethanol and paper.^{1–4}

The EnzChek® Ultra Amylase Assay Kit (E33651) provides a solution-based assay featuring the speed, high sensitivity, and convenience required for measuring amylase activity or for screening amylase inhibitors in a high-throughput format. This EnzChek kit contains a starch derivative — the DQ™ starch substrate — that is labeled with BODIPY® FL dye (Figure 1) to such a degree that the fluorescence is quenched. This substrate is efficiently degraded by amylase; digestion relieves the quenching and yields highly fluorescent fragments. The accompanying increase in fluorescence is proportional to amylase activity (Figures 2 and 3) and can be monitored with a fluorescence microplate reader or fluorometer, using standard fluorescein filters.

In tests using α -amylase from *Bacillus sp.* (Sigma A-6380) and 200 μ g/mL of the DQ starch substrate (30 minute incubation at room temperature), the EnzChek Ultra Amylase Assay Kit could be used to detect α -amylase activity down to a final concentration of 2×10^{-3} U/mL, where one unit is defined as the amount of enzyme required to liberate 1 mg of maltose from starch in 3 minutes at 20°C, pH 6.9.

Materials

Kit Contents

The EnzChek Ultra Amylase Assay Kit provides the following components:

- **DQ starch from corn, BODIPY FL conjugate** (Component A), five vials each containing ~1 mg of the lyophilized substrate
- **10X Reaction Buffer** (Component B), 10 mL of 0.5 M MOPS (pH 6.9)
- **Substrate solvent** (Component C), 1 mL of 50 mM sodium acetate (pH 4.0)
- **Fluorescence standard** (Component D), 0.5 mL of 1 mM BODIPY FL propionic acid in DMSO

The kit provides sufficient reagents for 500 assays using a 100 μ L assay volume in a 96-well microplate assay format.

Storage and Handling

Upon receipt, store the kit at room temperature, desiccated, protected from light. When stored properly, these reagents are stable for at least six months.

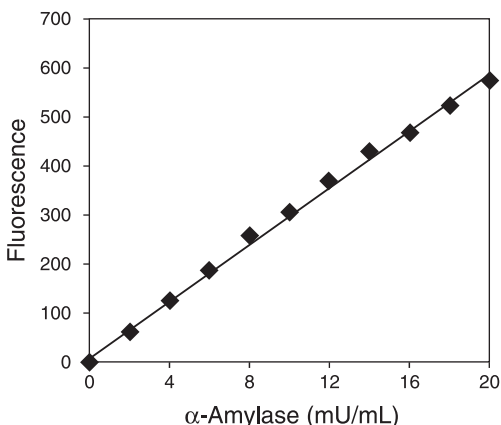


Figure 2. Assay of α -amylase from *Bacillus sp.* (Sigma A-6380) using the EnzChek *Ultra* Amylase Assay Kit. Reactions contained 200 μ g/mL DQ starch and the indicated amount of α -amylase in 100 mM MOPS (pH 6.9). After incubating at room temperature for 30 minutes, the fluorescence was measured using a microplate reader set for excitation at 485 ± 12 nm and emission detection at 520 ± 12 nm. The fluorescence of the blank sample (without enzyme) was subtracted from each value.

Experimental Protocol

The following procedures are designed for use with a 96-well fluorescence microplate reader. For use with a standard fluorometer, volumes must be increased accordingly.

Reagent Preparation

1.1 Dilute 10X reaction buffer to 1X. For example, take 2.5 mL of the 10X Reaction Buffer (Component B) and add 22.5 mL deionized water. This 25 mL of 1X reaction buffer is sufficient for at least 200 assays performed in a 100 μ L volume with \sim 5 mL excess for performing dilutions and preparing working solutions.

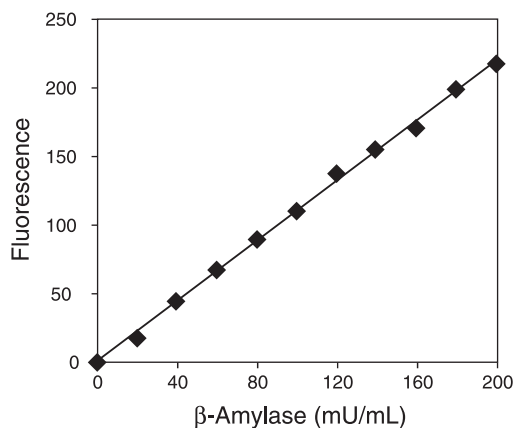


Figure 3. Assay of β -amylase from barley (Sigma A-7130) using the EnzChek *Ultra* Amylase Assay Kit. Reactions contained 200 μ g/mL DQ starch and the indicated amount of β -amylase in 100 mM sodium acetate buffer (pH 4.8). After incubating at room temperature for 30 minutes, the fluorescence was measured using a microplate reader set for excitation at 485 ± 10 nm and emission detection at 530 ± 15 nm. The fluorescence of the blank sample (without enzyme) was subtracted from each value.

1.2 Prepare a 1 mg/mL stock solution of the DQ starch substrate by dissolving the contents of one of the five vials containing the lyophilized substrate (Component A) in 100 μ L of 50 mM sodium acetate buffer (pH 4.0) (Component C). One vial of 1 mg/mL substrate solution is sufficient for one 96-well microplate. Vortex for about 20 seconds and leave at room temperature with occasional mixing for \sim 1–5 minutes, until dissolved. Next, add 900 μ L of an optimal amylase buffer (or 1X Reaction Buffer for analysis of α -amylase from *Bacillus sp.*). Mix well; store the substrate solution at room temperature, in the dark, until ready to use.

Note: This 1 mg/mL stock solution can be stored for a few days at 4°C, protected from light, but this is not recommended. Do not freeze the DQ starch stock solution as background fluorescence of the substrate may increase upon freezing and thawing.

1.3 Prepare an amylase standard curve. Dilute the amylase appropriately, using the optimal buffer for the chosen amylase. Include a blank sample that contains buffer and substrate only (no-enzyme blank). We recommend using *Bacillus sp.* α -amylase (Sigma A-6380) as a standard. Dilute the α -amylase in 1X reaction buffer to create a standard curve between 0 and 20 mU/mL, in triplicate.

1.4 Prepare several dilutions of the sample to be analyzed, using the optimal buffer for the enzyme (or 1X Reaction Buffer for *Bacillus sp.* α -amylase). Dilute the enzyme sufficiently to ensure that the activity is within the range of the standard curve.

1.5 If a fluorescence standard curve is required for reference, the 1 mM BODIPY FL fluorescence standard (Component D) can be serially diluted in the buffer of choice. The fluorescent standard can be used as a control to assess instrument-to-instrument variation and day-to-day variation in single-instrument performance.

Assay for Amylase Activity

The following protocol describes the amylase assay in a total volume of 100 μ L.

2.1 Prepare a 200 μ g/mL working solution of the DQ substrate by performing a five-fold dilution of the 1 mg/mL substrate solution (prepared in step 1.2). For example, take the 1 mL of the 1 mg/mL substrate stock solution and add 4 mL of the optimal reaction buffer (or 1X Reaction Buffer, as required).

2.2 If a fluorescence standard curve is required for reference, add 50 μ L of the BODIPY FL fluorescence standard curve samples (prepared in step 1.5) into wells containing 50 μ L reaction buffer.

2.3 To create an enzyme standard curve, mix the amylase standard curve samples and blank. Pipet 50 μ L of this solution, in duplicate or triplicate, into microplate wells.

2.4 Add 50 μ L of the samples to be analyzed, in duplicate or triplicate, to the wells.

2.5 Mix the 200 µg/mL substrate solution and quickly add the substrate, with mixing, to all wells containing the enzyme standard curve and enzyme test samples (best results will be achieved by using a multichannel pipettor).

2.6 Incubate the samples at room temperature, protected from light, for an appropriate time (10–30 minutes). For *Bacillus sp.* α-amylase, 30 minutes is recommended for maximum sensitivity.

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

2.7 Measure the fluorescence intensity in a fluorescence microplate reader equipped with standard fluorescein filters. Digestion products from the DQ starch substrate have an absorption maximum at ~505 nm and a fluorescence emission maximum at ~512 nm.

2.8 For each data point, correct for background fluorescence by subtracting the value derived from the no-enzyme blank. Plot these corrected fluorescence values against the enzyme concentration to create a standard curve. Use the linear range of the standard curve to determine the concentration of amylase in the test sample.

References

1. *Medicine (Paris)* 55, 269 (1976); 2. *Clin Chem* 21, 57 (1976); 3. *Clin Chem* 30, 387 (1984); 4. *Starch/Stärke* 35, 169 (1983).

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

Cat #	Product Name	Unit Size
E33651	EnzChek® <i>Ultra</i> Amylase Assay Kit *500 assays*	1 kit

Contact Information

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.

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