

EnzChek[®] Elastase Assay Kit (E-12056)

Quick Facts

Storage upon receipt:

- -20°C
- Protect from light

Ex/Em of digestion product: 505/515 nm

Note: Avoid freeze-thaw cycles after reconstituting

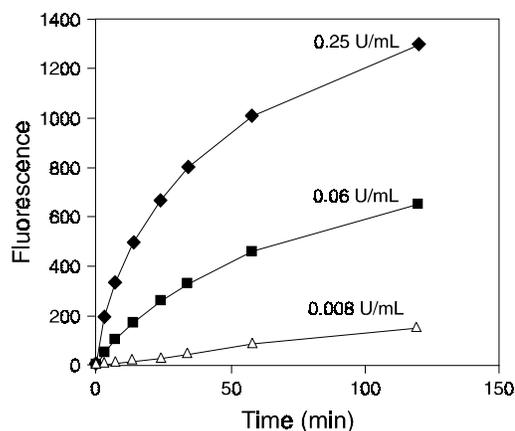


Figure 2. Kinetics of the EnzChek Elastase reaction. Elastase at 0.008, 0.06 and 0.25 U/mL was incubated for the indicated time periods with 25 µg/mL DQ elastin substrate. Fluorescence was measured as in Figure 1. Background fluorescence, determined for a no-enzyme control reaction, has been subtracted from each value.

Introduction

Molecular Probes' EnzChek[®] Elastase Assay Kit provides the speed, high sensitivity and convenience required for measuring elastase activity or for screening inhibitors in a high-throughput format. The EnzChek kit contains DQ[™] elastin — soluble bovine neck ligament elastin that has been labeled with our BODIPY[®] FL dye such that the conjugate's fluorescence is quenched. The non-fluorescent substrate can be digested by elastase or other proteases to yield highly fluorescent fragments (Figure 1). The resulting increase in fluorescence can be monitored with a fluorescence microplate reader, minifluorometer or

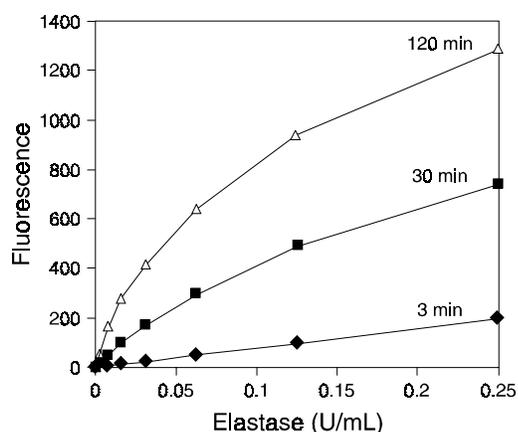


Figure 1. Assay of porcine pancreatic elastase using the EnzChek Elastase Assay Kit. Aliquots of DQ elastin at final concentrations of 25 µg/mL were incubated with various concentrations of elastase in a 96-well microplate. After incubation times of 3, 30 and 120 minutes, fluorescence was measured in a fluorescence multi-well plate reader set for excitation at 485 ± 10 nm and emission detection at 530 ± 15 nm. Background fluorescence (250 arbitrary units, in this experiment) has been subtracted from each value.

standard fluorometer. Digestion products from the DQ elastin substrate have absorption maxima at ~505 nm and fluorescence emission maxima at ~515 nm. Using a two hour incubation period and a fluorescence microplate reader equipped with standard fluorescein filters, the kit can detect the activity of elastase from porcine pancreas down to a final concentration of 5×10^{-3} U/mL (40 ng protein/mL). Because the assay is continuous, kinetic data can be obtained easily (Figure 2). Furthermore, because the fluorescence of the BODIPY FL dye is pH insensitive between pH 3 and 9, the assay can be performed under a variety of buffer conditions. Please note that DQ elastin is also digested by proteases other than elastase. The kit includes a selective inhibitor of elastase, *N*-methoxysuccinyl-Ala-Ala-Pro-Val-chloromethyl ketone, which can be used to help confirm the identity of the protease responsible for substrate digestion.^{1,2} Peptide chloromethyl ketones react with serine proteases to irreversibly inhibit these enzymes. It is the peptide portion that targets the inhibitor to the active site and the better the "fit" of the peptide within the active site, the faster the inactivation occurs. Powers and colleagues¹ have demonstrated that, at low concentrations (~10–50 µM), *N*-methoxysuccinyl-Ala-Ala-Pro-Val-chloromethyl ketone inhibits porcine pancreatic elastase and human leukocyte elastase at relatively fast rates, whereas this peptide chloromethyl ketone does not inhibit human leukocyte cathepsin G. Thus, at suitable concentrations, this peptide chloromethyl ketone can be used as a selective inhibitor of elastase. This compound can also be used as a control inhibitor when utilizing the EnzChek Elastase Assay Kit for screening for elastase inhibitors.

Materials

Kit Contents

- **DQ elastin from bovine neck ligament, BODIPY FL conjugate** (Component A), three vials each containing 1 mg substrate lyophilized from 1 mL phosphate-buffered saline, pH 7.2 (PBS)
- **10X Reaction Buffer** (Component B), ~28 mL of 1 M Tris-HCl, pH 8.0, containing 2 mM sodium azide
- **Elastase from pig pancreas** (Component C), 50 units, where one unit is defined as the amount of enzyme necessary to solubilize 1 mg of elastin in 20 minutes at pH 8.8 and 37°C
- **N-Methoxysuccinyl-Ala-Ala-Pro-Val-chloromethyl ketone** (Component D), MW = 503, inhibitor of elastase, 500 µg

Each kit provides sufficient reagents for approximately 600 assays using a fluorescence microplate reader (200 assays from each vial of DQ elastin substrate).

Storage and Handling

Upon receipt, the kit should be stored at -20°C, protected from light. Allow reagents to warm to room temperature before opening vials. When stored properly, these reagents are stable for at least six months.

Experimental Protocol

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

Reagent Preparation

1.1 Prepare a 1.0 mg/mL stock solution of the DQ elastin substrate by adding 1.0 mL of deionized water (dH₂O) directly to one of the three vials containing the lyophilized substrate. Mix thoroughly to dissolve. Reconstituted DQ elastin may be stored for approximately one week at 4°C, protected from light, with the addition of sodium azide to a final concentration of 2 mM. For longer storage, divide into aliquots and freeze at -20°C. Because background fluorescence of the substrate may increase upon freezing and thawing, **AVOID REPEATED FREEZING AND THAWING OF DQ ELASTIN SOLUTIONS.**

1.2 Prepare 1X Reaction Buffer. Dilute 6 mL of the 10X Reaction Buffer in 54 mL dH₂O. This 60 mL volume of working 1X Reaction Buffer is sufficient for at least 200 assays, each containing a volume of 200 µL, with sufficient excess for performing dilutions and preparing working solutions.

1.3 Prepare a 100 µg/mL working solution of the DQ elastin substrate by diluting the DQ elastin stock solution (prepared in step 1.1) tenfold in 1X Reaction Buffer. A volume of 50 µL will be used for each 200 µL reaction volume.

1.4 If using the porcine pancreatic elastase, prepare a 100 U/mL stock solution by dissolving the contents of the vial (Component C) in 0.5 mL dH₂O. Reconstituted elastase can be frozen in aliquots and stored at -20°C for at least six months without significant loss of activity.

1.5 If desired, prepare a 10 mg/mL stock solution of the elastase inhibitor, N-methoxysuccinyl-Ala-Ala-Pro-Val-chloromethyl ke-

tone, by adding 50 µL of anhydrous dimethylsulfoxide directly to the vial of inhibitor (Component D). This solution can be stored frozen at -20°C for at least three months.

Assays for Elastase Activity

2.1 Pipet 50 µL of 1X Reaction Buffer (prepared in step 1.2) into each assay well. If an inhibitor is to be used, see *Assay of Elastase in the Presence of Inhibitors*, below.

2.2 Add 50 µL of 100 µg/mL DQ elastin working solution (prepared in step 1.3) to the wells. Pipet up and down to mix. This amount of DQ elastin will give a final substrate concentration of 25 µg/mL.

2.3 Dilute the enzyme of interest in 1X Reaction Buffer to prepare a 2X enzyme working solution. We recommend trying a number of different enzyme dilutions.

2.4 Add 100 µL of the diluted enzyme, or 100 µL of 1X Reaction Buffer as a negative control, to the substrate samples and mix to begin the reactions. If a positive control is desired, use 100 µL of diluted porcine pancreatic elastase. The elastase stock solution prepared in step 1.4 should be diluted to an appropriate concentration in 1X Reaction Buffer. We have found that elastase at a final concentration of 0.1–0.2 U/mL is reasonable for a 30 minute incubation period. For shorter incubation periods, use more enzyme; for longer incubation periods, use less (see Figures 1 and 2 for guidance).

2.5 Incubate the samples at room temperature, protected from light, for an appropriate time. Because the assay is continuous (not terminated), fluorescence may be measured at multiple time points.

2.6 Measure the fluorescence intensity in a fluorescence microplate reader equipped with standard fluorescein filters.

2.7 For each time point, correct for background fluorescence by subtracting the value derived from the no-enzyme control.

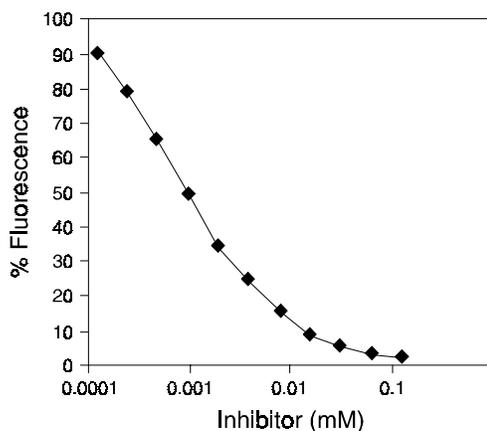


Figure 3. Inhibition of elastase by N-methoxysuccinyl-Ala-Ala-Pro-Val-chloromethyl ketone. DQ elastin substrate at 25 µg/mL, elastase at 0.5 U/mL and increasing amounts of the inhibitor were incubated together for 2 hours. Fluorescence was measured as described in Figure 1. All values were corrected for background fluorescence and expressed relative to the fluorescence obtained in the absence of inhibitor.

Assay of Elastase in the Presence of Inhibitors

The included elastase inhibitor, *N*-methoxysuccinyl-Ala-Ala-Pro-Val-chloromethyl ketone, can be used as a control inhibitor for use when screening potential elastase inhibitors (Figure 3). Since DQ elastin is digested by proteases other than elastase, this inhibitor can also be used to help determine whether or not the observed proteolytic activity is the result of elastase.^{1,2} Below we provide a sample protocol illustrating the use of an inhibitor with the EnzChek Elastase Assay Kit. In actual practice, incubation times, enzyme concentrations and inhibitor concentrations will have to be optimized for the particular experimental conditions. Specifically, if using the inhibitor to confirm that elastase is responsible for the proteolytic activity, the inhibitor concentration and time of any preincubation with the enzyme-containing sample will have to be optimized such that elastase is selectively inhibited and that the inhibition is of the desired magnitude.

3.1 Dilute the inhibitor of interest in 1X Reaction Buffer (prepared in step 1.2) to prepare a 4X inhibitor working solution. A 50 μ L volume of inhibitor will be used for each 200 μ L reaction. For *N*-methoxysuccinyl-Ala-Ala-Pro-Val chloromethyl ketone, see Figure 3 for a guide to an appropriate *final* inhibitor concentration. Include a no-inhibitor control for all enzymes being assayed.

3.2 Add 50 μ L of the diluted inhibitor (or no-inhibitor control) to each assay well.

3.3 Next, add 50 μ L of 100 μ g/mL DQ elastin working solution (prepared in step 1.3) to each assay well. Note: This addition of the DQ elastin substrate must be delayed until after step 3.4, if a preincubation of inhibitor and elastase is desired.

3.4 Dilute the enzyme of interest, and porcine pancreatic elastase if desired (see step 2.4), in 1X Reaction Buffer. Add 100 μ L of the diluted enzyme, or 100 μ L of 1X Reaction Buffer as a blank, to the sample wells preloaded with substrate and inhibitor.

3.5 Incubate the samples at room temperature, protected from light, for an appropriate time, e.g. 1–2 hours. Because the assay is continuous (not terminated), fluorescence may be measured at multiple time points.

3.6 Measure the fluorescence intensity in a fluorescence microplate reader equipped with standard fluorescein filters.

3.7 For each time point, correct for background fluorescence by subtracting the values derived from the no-enzyme control.

References

1. Biochim Biophys Acta 485, 156 (1977);
2. Biochemistry 25, 5414 (1986).

Product List

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

Cat #	Product Name	Unit Size
E-12056	EnzChek® Elastase Assay Kit *600 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 Leiden, the Netherlands. All others should contact our Technical Assistance Department in Eugene, Oregon.

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Molecular Probes, Inc.

PO Box 22010, Eugene, OR 97402-0469
Phone: (541) 465-8300 • Fax: (541) 344-6504

Customer Service: 7:00 am to 5:00 pm (Pacific Time)
Phone: (541) 465-8338 • Fax: (541) 344-6504 • order@probes.com

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Technical Assistance: 8:00 am to 4:00 pm (Pacific Time)
Phone: (541) 465-8353 • Fax: (541) 465-4593 • tech@probes.com

Molecular Probes Europe BV

PoortGebouw, Rijnsburgerweg 10
2333 AA Leiden, The Netherlands
Phone: +31-71-5233378 • Fax: +31-71-5233419

Customer Service: 9:00 to 16:30 (Central European Time)
Phone: +31-71-5236850 • Fax: +31-71-5233419
eurorder@probes.nl

Technical Assistance: 9:00 to 16:30 (Central European Time)
Phone: +31-71-5233431 • Fax: +31-71-5241883
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