SensoLyte® Green Elastase Assay Kit
Fluorimetric

Catalog #	**72178**
Kit Size	500 Assays (96-well plate)

- ***Optimized Performance:*** This kit is optimized to detect elastase activity.
- ***Enhanced Value:*** It provides ample reagents to perform 500 assays in a 96-well format.
- ***High Speed:*** The entire process can be completed in one hour
- ***Assured Reliability:*** Detailed protocol and references are provided.

Kit Components, Storage and Handling

Component	Description	Quantity
Component A	5-FAM/QXL™ 520 labeled elastin, Ex/Em=490 /520 nm upon cleavage	250 µL
Component B	Elastase, porcine pancreas	0.4 mg/mL, 100 µL (5 vials)
Component C	2X Assay Buffer	30 mL
Component D	Elastase inhibitor (MeOSuc-Ala-Ala-Pro-Val-CMK)	10 mM, 50 µL

Other Materials Required (but not provided)

- 96-well microplate: Black, flat bottom 96-well plate with non-binding surface.
- Fluorescence microplate reader: Capable of detecting emission at 520 nm with excitation at 490 nm.

Storage and Handling

- Store all kit components at -20°C
- Protect Component A from light and moisture.
- Component C can be stored at room temperature for convenience.

Introduction

Elastases catalyze the hydrolysis of elastin, the chief constituent of elastic fibers that together with collagen determines the mechanical properties of connective tissue. Elastases have many other natural substrates these include proteoglycans, collagens and fibronectin.[1-4] Implicated in many diseases such as pulmonary emphysema, cystic fibrosis, infections, inflammation and atherosclerosis, elastases are considered important drug targets.[5]

The SensoLyte® Green Elastase Assay Kit uses natural substrate elastin labeled with the 5-FAM fluorophore and the QXL™ 520 quencher. Proteolytic cleavage of labeled elastin yields brightly green fluorescence, which can be continuously monitored at excitation/emission= 488 nm/520 nm. Increase in fluorescence intensity is directly proportional to elastase activity. This kit does not require any separation steps and can be used to continuously measure the kinetics of elastase activity.

Protocol

Note 1: For standard curve, please refer to Appendix II (optional).
Note 2: Please use protocol A or B based on your needs.

Protocol A. Screening elastase inhibitors using purified enzyme.

1. Prepare working solutions.

Note: Thaw all kit components to room temperature before starting the experiments.

1.1 1X Assay buffer: For one 96-well plate, add 5 mL of 2X assay buffer (Component C) to 5 mL of deionized water.

1.2 Elastase substrate solution: Dilute elastase substrate (Component A) 100-fold in assay buffer. Prepare fresh substrate solution for each experiment.

Table 1. Elastase substrate solution for one 96-well plate (100 assays).

Components	**Volume**
Elastase substrate (100 X, Component A)	50 µL
1X Assay buffer	4.95 mL
Total volume	5 mL

Note: Dilute substrate according to the amount needed. Save the rest of the 100x substrate solution for future experiments.

1.3 Elastase diluents: Dilute one vial of the elastase enzyme (Component C) 40-fold in 1X assay buffer (refer to Table 2). The amount of enzyme is enough for a full 96-well plate. If not using the entire plate, adjust the amount of enzyme to be diluted accordingly.

Table 2. Elastase enzyme solution for one 96-well plate (100 assays).

Components	**Volume**
Elastase enzyme (Component C)	100 µL
1X Assay buffer	3.9 mL
Total volume	4 mL

Note: Prepare elastase diluents immediately before use. Do not vortex the enzyme solution. Prolonged storage or vigorous agitation of the diluted enzyme will cause denaturation. Store the enzyme solution on ice.

1.4 Elastase inhibitor (MeOSuc-Ala-Ala-Pro-Val-CMK): Dilute 10 mM inhibitor solution (Component D) 10-fold in assay buffer to get a concentration of 1 mM. Add 10 µl of the diluted compound into each of the inhibitor control well.

2. Set up the enzymatic reaction.

2.1 Add test compounds and diluted enzyme solution to the microplate wells. For one well of a 96-well plate, the suggested volume of enzyme solution is 40 µL and 10 µL of test compound.

2.2 Simultaneously set up the following control wells, as deemed necessary:

- Positive control contains the enzyme without test compound.
- Inhibitor control contains elastase enzyme and inhibitor.
- Vehicle control contains elastase and vehicle used in delivering test compound (e.g. DMSO, concentration not to exceed 1%).
- Test compound control contains assay buffer and test compound. Some test compounds have strong autofluorescence and may give false results.
- Substrate control contains 1X assay buffer.

2.3 Using the assay buffer, bring the total volume of all controls to 50 µL.

2.4 Optional: Pre-incubate the plate for 10 min. at assay temperature. Any temperature (*the assay temperature*) from room temperature to 37°C may be used, as long as the subsequent incubations are performed at the same temperature.

3. Run the enzymatic reaction.

3.1 Add 50 µL of elastase substrate solution into each well. For best accuracy, it is advisable to have the substrate solution equilibrated to the assay temperature. Mix the reagents completely by shaking the plate gently for 30 sec.

3.2 Measure fluorescence signal:

- For kinetic reading: Immediately start measuring fluorescence intensity at Ex/Em=490 nm/520 nm continuously and record data every 5 min. for 30 to 60 min.
- For end-point reading: Incubate the reaction for 30 to 60 min. Keep plate from direct light, then measure fluorescence intensity at Ex/Em=490 nm/520 nm.

3.3 For methods of data analysis: Refer to Appendix I.

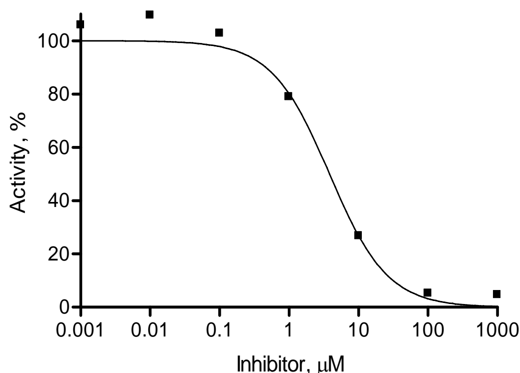

Figure 1. Inhibition of elastase activity by MeOSuc-Ala-Ala-Pro-Val-CMK peptide inhibitor was measured using SensoLyte® Green Elastase activity Assay Kit.

Protocol B. **Measuring elastase activity in biological samples.**

1. **Prepare working solutions.**

   Note: Thaw all kit components to room temperature before starting the experiments.

   1.1 Elastase substrate solution: Dilute elastase substrate (Component A) 100-fold in assay buffer. Prepare fresh substrate solution for each experiment.

   Table 1. Elastase substrate solution for one 96-well plate (100 assays).

Components	Volume
Elastase substrate (100 X, Component A)	50 µL
2X Assay buffer (Component C)	4.95 mL
Total volume	5 mL

   Note: Dilute substrate according to the amount needed. Save the rest of the 100x substrate solution for future experiments.

   1.2 Elastase diluent: If using elastase as a positive control, dilute the elastase enzyme (Component C) 50-fold in 1X assay buffer. Add 50 µL of the diluted enzyme into each of the positive control well.

   Note 1: Dilute elastase according to the amount needed.

   Note 2: Prepare elastase diluents immediately before use. Do not vortex the enzyme solutions. Prolonged storage or vigorous agitation of the diluted enzyme will cause denaturation. Store the enzyme solution on ice.

2. **Set up the enzymatic reaction**

   2.1 Add 50 µl of elastase containing sample.

   2.2 Simultaneously establish the following control wells

   - Positive control contains elastase positive sample or purified active elastase.
   - Substrate control contains deionized water.

   2.3 Bring the total volume of all controls to 50 µL.

   2.4 Optional: Pre-incubate the plate for 10 min. at assay temperature. Any temperature (*the assay temperature*) from room temperature to 37°C may be used, as long as the subsequent incubations are performed at the same temperature.

3. **Run the enzymatic reaction.**

   3.1 Add 50 µL of elastase substrate solution into each well. For best accuracy, it is advisable to have the substrate solution equilibrated to the assay temperature. Mix the reagents completely by shaking the plate gently for 30 sec.

   3.2 Measure fluorescence signal:

   - For kinetic reading: Immediately start measuring fluorescence intensity at Ex/Em=490 nm/520 nm continuously and record data every 5 min. for 30 to 60 min.
   - For end-point reading: Incubate the reaction for 30 to 60 min. Keep plate from direct light, then measure fluorescence intensity at Ex/Em=490 nm/520 nm.

   3.3 For methods of data analysis: Refer to Appendix I.

Appendix I. Data Analysis

- The fluorescence reading from the substrate control well is used as the background fluorescence. This background reading should be subtracted from the readings of the other wells containing substrate. All fluorescence readings are expressed in relative fluorescence units (RFU).
- For kinetics analysis:
    - Plot data as RFU versus time for each sample. To convert RFUs to the concentration of the product of the enzymatic reaction, please refer to Appendix II for establishing a fluorescence reference standard.
    - Determine the range of initial time points during which the reaction is linear. Typically, the first 10-15% of the reaction will be the optimal range.
    - Obtain the initial reaction velocity (V_o) in RFU/min by determining the slope of the linear portion of the data plot.
    - A variety of data analyses can be done, e.g., determining inhibition %, EC_{50}, IC_{50}, K_m, K_i, etc.
- For endpoint analysis:
    - Plot data as RFU versus concentration of test compounds.
    - A variety of data analyses can be done, e.g., determining inhibition %, EC_{50}, IC_{50}, etc.

References

1. Kafienah, W. et al. *Biochem J.* **330**, 897 (1998).
2. Carlo, L. et al. *J. Biol. Chem.* **255**, 12006 (1980).
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5. Bieth, JG. *J. Soc. Biol.* **195**, 173 (2001).

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