SensoLyte® AMC Caspase Substrate Sampler Kit *Fluorimetric*

Revision # 1.1	*Last updated:2/6/17*
Catalog #	**71121**
Kit Size	500 Assays (96-well plate)

- ***Convenient Format:*** All essential assay components are included.
- ***Optimized Performance:*** Optimal conditions for assaying the activity of caspase.
- ***Enhanced Value:*** Less expensive than the sum of individual components.
- ***High Speed:*** Minimal hands-on time.
- ***Assured Reliability:*** Detailed protocol and references are provided.

Kit Components, Storage and Handling

Component	Description	Quantity
Component A	8 AMC-based caspase substrates. Refer to **Appendix I, table 1** for peptide sequence. Ex/Em=354nm/442 nm upon cleavage	10 mM DMSO solution, 70 µL each
Component B	AMC, fluorescence reference standard Ex/Em=354nm/442 nm	10 mM DMSO solution, 20 µL
Component C	Assay Buffer	100 mL
Component D	DTT	1 M, 1 mL X 2 vials
Component E	10X Lysis Buffer	20 mL

Other Materials Required (but not provided)

- 96-well microplate: Black, flat-bottom microplates with non-binding surface.
- Microplate reader: Capable of detecting absorbance at 405 nm or 415 nm.

Storage and Handling

- Store all kit components at -20°C
- Protect Components A and B from light

Introduction

Apoptosis is a programmed, cell-autonomous death process. It is involved in a variety of physiological and pathological events[1], ranging from normal fetal development to diseases, such as cancer[2], organ failure and neurodegenerative diseases. During apoptosis, caspases execute the disassembly of the cellular components by proteolytic cleavage of a variety of substrates, such as poly-(ADP ribose) polymerase (PARP)[3], DNA-dependent protein kinase (DNA-PK), topoisomerases, and protein kinase C (PKC)δ.[4] At least ten caspases have been discovered. Some of caspases identify and cleave a specific peptide substrate, while others recognize the same peptide substrate[4].

The SensoLyte® AMC Caspase Substrate Sampler Kit contains 8 AMC-based peptide substrates (**Appendix I**, Table 1) as fluorogenic indicators for assaying caspase activities. This kit provides a convenient platform for profiling substrate specificity of caspases and optimizing assay condition for caspases. AMC-based substrates are widely used to monitor caspase activity and can be monitored at excitation/emission = 354 nm/442 nm

Protocol

Note 1: Warm all the kit components until thawed to room temperature before starting the experiments.
Note 2: For standard curve, please refer to Appendix II (optional).
Note 3: You can formulate your own assay buffer and design your own assay experiment. For your convenience, this kit includes an assay buffer and the following suggested assay procedures.

1. Prepare working solutions.

1.1 Assay buffer: Add 1 M DTT (Component D) 20 µL per mL of assay buffer (Component C).

1.2 Caspase substrate solution: Dilute caspase substrate (Component A) 1:100 in assay buffer.

1.3 Prepare caspase diluent:
If you use purified caspase: Dilute caspase to a concentration of 50 to 10 nM in assay buffer (Component C).

If you use caspase-containing biological sample: Refer to **Appendix III** for sample preparation.

Note: Warm up the caspase samples to room temperature before proceeding with the following enzymatic reaction.

2. Initiate the enzymatic reaction.

2.1 Add 50 µL/well caspase diluent to 96-well plate.

2.2 Set up the following control: Negative control contains 50 µL of caspase dilution buffer (Component E) if purified enzyme is used, or 50 µL 1X lysis buffer (diluted Component F) if biological sample is used.

2.3 Add 50 μL/well caspase substrate solution to a 96-well plate. Mix the reagents by shaking the plate gently for 30 seconds. Immediately start measuring fluorescence intensity at Ex/Em=354 nm/442 nm continuously and record data every 5 minutes for 30 minutes.

Appendix I

Table 1: Caspase substrate sequences.

Substrate No.	Substrate Name	Substrate Sequence
SB1	Caspase-1 substrate	Ac-YEVD-AMC
SB2	Caspase-1 substrate	Ac-WEHD-AMC
SB3	Caspase-1/4 substrate	Ac-YVAD-AMC
SB4	Caspase-3 substrate	Ac-DMQD-AMC
SB5	Caspase-3/7 substrate	Ac-DEVD-AMC
SB6	Caspase-6 substrate	Ac-VEID-AMC
SB7	Caspase-8 substrate	Ac-IETD-AMC
SB8	Caspase-9 substrate	Ac-LEHD-AMC

Appendix II: Instrument calibration

- AMC fluorescence reference standard: Dilute 10 mM AMC (Component B) to 60 μM in deionized water. Do 2-fold serial dilutions to obtain 30, 15, 7.5, 3.75, 1.88, 0.94 μM AMC solutions, include a water blank. Add 50 μL/well of the serially diluted AMC solutions from 60 μM to 0 μM into the reference standard wells (refer to Table 1).
- Add 50 μL/well of assay buffer. Mix the reagents by shaking the plate gently for 3 to 5 seconds.
- Measure the fluorescence intensity of reference standards at Ex/Em=354 nm/442 nm. Adjust the sensitivity of the microplate reader until satisfactory signals can be read. Use the same setting of sensitivity in all the enzymatic reaction.
- Plot AMC fluorescent reference standard as RFU (relative fluorescent unit) versus concentration as **Figure 1**.

  Note: The final concentrations of the AMC reference standard solutions are 30, 15, 7.5, 3.75, 1.88, 0.94, 0.47 and 0 μM. The resulting reference standard curve is used to calibrate for the variation of different instruments and different batches of experiments. It also can be served as an indicator of the amount of caspase enzymatic reaction final product.

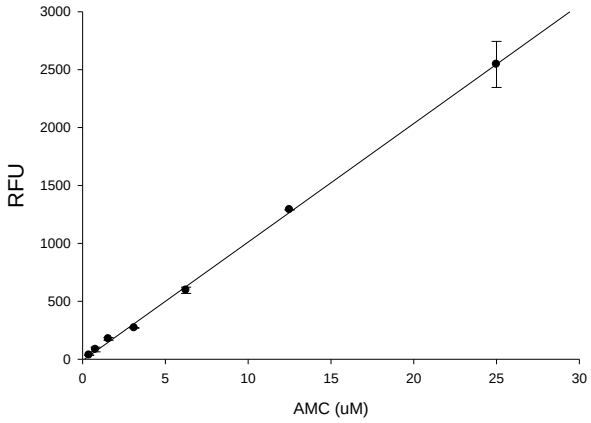

Figure 1. AMC reference standard calibration curve.

AMC was diluted in assay buffer. 100 µL of AMC at each concentration was added into a 96-well microplate. The fluorescence signal was measured by a fluorescence microplate reader (FLx800, Bio-Tek Instruments) with a filter set of Ex/Em=360±40 nm/460±40 nm. (mean ± S.D.; n= 2 independent samples).

Appendix III

1. Prepare caspase-containing sample from cell extract.

- Induce apoptosis in cell culture with desired method.
- Prepare 1X lysis buffer by adding 1 mL of 10X lysis buffer (Component F) to 9 mL of deionized water.
- Suspension cells are collected by centrifugation at 500 X g for 5 minutes. For adherent cells, simply aspirate the growth medium.
- Add an appropriate amount of 1X lysis buffer to cells or cell pellet, e.g. 300 µL 1X lysis buffer for one well of 6-well plate. Scrape off the adherent cells or re-suspend the cell pellet, and then collect the cell suspension in a microcentrifuge tube.
- Rotate the cell suspension on a rotating apparatus for 30 min at 4°C.
- Centrifuge the cell suspension at 2500 X g for 10 min at 4°C.

2. Prepare caspase-containing sample from tissue.

- Prepare 1X lysis buffer by adding 1 mL of 10X lysis buffer (Component F) to 9 mL of deionized water.
- Tissues samples should be homogenized in 1X lysis buffer, and then centrifuged for 15 min at 10,000x g at 4°C. The supernatant, which contains caspase, can be frozen at -70 °C until use.

References

1. Thornberry, N. A. et al. *Science* **281**, 1312-1316 (1998).
2. Reed, J. C. *J. Clin. Oncol.* **17**, 2941-2953 (1999).
3. Lazebnik, Y. A. et al., *Nature* **371**, 346-347 (1994).
4. Villa, P. et al., *Trends Biochem. Sci.* **22**, 388-393 (1997