MeO-Succ-Arg-Pro-Tyr-AMC

Catalog number: 13460
Unit size: 5 mg

Component	Storage	Amount (Cat No. 13460)
MeO-Succ-Arg-Pro-Tyr-AMC	Freeze (< -15 °C), Minimize light exposure	1 vial (5 mg)

OVERVIEW

MeO-Succ-Arg-Pro-Tyr-AMC is a sensitive fluorogenic substrate for chymotrypsin-like proteases. The non-fluorescent substrate generates a bright blue fluorescent AMC product that has an emission spectra that can be easily detected with the DAPI filter set. It has been used for monitoring the protease activity of stratum corneum chymotryptic enzyme (SCCE).

KEY PARAMETERS

Fluorescence microplate reader

Cutoff	435 nm
Emission	470 nm
Excitation	360 nm
Recommended plate	Solid black

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles

1. MeO-Succ-Arg-Pro-Tyr-AMC is provided as a lyophilized powder. To prepare a 10 mM stock solution, dissolve 1 mg of the compound in 122 µL of DMSO.

PREPARATION OF WORKING SOLUTION

1. Prepare a substrate working solution at a final concentration of 20–50 µM using the reaction buffer (100 mM Tris-HCl, pH 8.0, containing 0.4 M NaCl and 0.1% Triton X-100).

   Note: Prepare the MeO-Succ-Arg-Pro-Tyr-AMC working solution immediately before each experiment, and protect it from light.

SAMPLE EXPERIMENTAL PROTOCOL

Well	Volume
STD1-STD7	50 µL AMC serial dilutions (0.078 µM-10 µM)
BL	50 µL reaction buffer
Test Sample	50 µL
Sample Buffer Control	50 µL of the buffer that the test sample is in

1. Prepare the AMC standards (STD1–STD7), blank controls (BL), and test samples according to the layout specified in the table above.
2. Add 50 µL of Working Solution to each well designated for Test Samples and Sample Buffer Control. For STD1-STD7 and BL wells, add 50 µL reaction buffer.
3. Incubate the samples at room temperature for 30–60 minutes, protected from light. Or, monitor the reaction in real time using kinetic mode
4. Monitor the fluorescence at Ex/Em= 360/470 nm (cutoff=435 nm).

EXAMPLE DATA ANALYSIS AND FIGURES

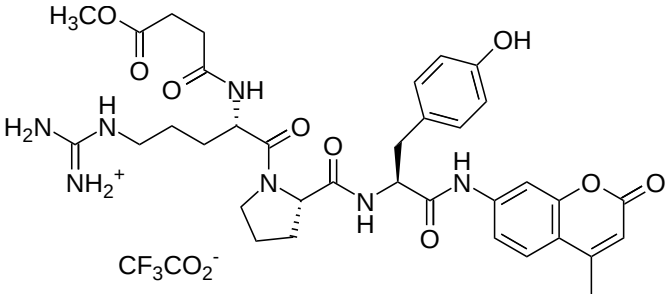

Figure 1. Chemical structure for MeO-Succ-Arg-Pro-Tyr-AMC

Data Analysis

Enzyme Activity = Delta Con. of AMC (µM) / Delta T (min)

- Delta Con. of AMC (uM): The amount of AMC generated by the DPP4 assay between 0 min and 30 min determined from the standard curve.
- Delta Con. of AMC (uM) = Con. $_{final}$– Con.$_{initial}$ (uM)
- Delta T (min): Reaction Time = T_{final}– $T_{initial}$ (minutes)

Note: 1 unit (U) is the amount of enzyme that catalyzes the reaction of 1 µmol of substrate per minute.

- mU/mL = mole/min/mL=µM/min

DISCLAIMER

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