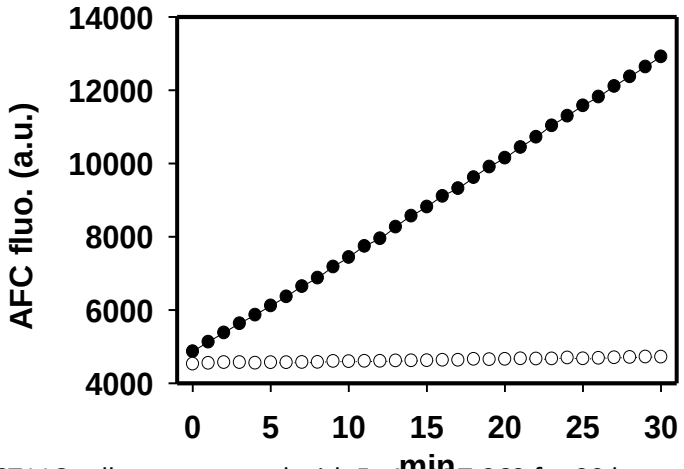
Ac-Leu-Glu-His-Asp-AFC (Ac-LEHD-AFC)

Cat. # G5200, G5201

Also Known as:	Ac-LEHD-AFC; Caspase 9 substrate
Cas#:	210345-03-2
MW (no tag):	765.7 Da
Formula:	C33H38F3N7O11
Source:	Synthetic
Tag:	N/A
Stock Buffer:	Powder
Solubility:	Soluble in DMSO
Concentration:	N/A
Quality Assurance:	> 95% by HPLC
Description:	Ac-LEHD-AFC is a fluorogenic substrate of caspase-9. Working concentration of this substrate is 25-50 µM. The released AFC (7-Amino-4-trifluoromethylcoumarin) fluorescence can be detected by a fluorimeter or plate reader using excitation/emission wavelengths at 400 nm/505 nm, respectively.
Storage:	Eligible for room temperature shipping. Store at -20°C upon receiving; avoid multiple freeze-thaw cycles after dissolving in DMSO. Protect from light.
Protocol:	**Users are recommended to optimize conditions based on their needs.** 1. Briefly spin the product packing tube using a desktop centrifuge to pellet the powder before removing the cap. 2. Prepare a 25 mM substrate stock in DMSO: add 0.26 mL DMSO to 5 mg Ac-LEHD-AFC powder or 1.3 mL DMSO to 25 mg Ac-LEHD-AFC powder. 3. Prepare 1X Reaction Buffer: 20 mM Tris, pH 7.6 at 4 ^{0}C, 150 mM NaCl, and 2 mM DTT. 4. Prepare 2X substrate (100 µM): add 20 µl substrate stock prepared in step 2 to 5 ml warmed (37 ^{0}C) 1X reaction buffer. Briefly vortex to dissolve. 5. Mix 50 µl apoptotic cell/tissue lysates (using more or less depending on capase amounts in your samples) or recombinant caspases with 50 µl 2X substrate prepared in step 4. Incubate at 37 ^{0}C for 10-60 min (users should optimize incubation time). 6. Record AFC fluorescence using a fluorometer or a plate reader using excitation/emmission wavelengths at 400 nm/505 nm, respectively. 7. Alternatively, released AFC fluorescence can be recorded continuously using a kinetic mode when the substrate is mixed with samples. 8. Reading from a control reaction should be subtracted as the background signal. An appropriate control reaction is: apoptotic cell/tissue lysate + substrate + a caspase inhibitor (such as 20 µM Z-VAD-FMK).

Image:



HCT116 cells were treated with 5 uM ABT-263 for 20 hours to activate intrinsic apoptosis. 50 ug cell lysates were incubated with (open circles) or without (solide circles) 20 uM Z-VAD-FMK (a pan caspase inhibitor) for 10 min at 37 ^{0}C, and then 25 uM Ac-LEHD-AFC was added to initiate the reaction. AFC fluorescence was recorded with a plate reader using excitation/emission filter sets at 400/508 nm, respectively.

References:

Poreba M., et al., Cold Spring Harb Perspect Bio. 2013, a008680.