Ac-Tyr-Val-Ala-Asp-AFC (Ac-YVAD-AFC)

Cat. # G5400, G5401

Also Known as:	Ac-YVAD-AFC; Caspase-1/4 substrate
Cas#:	219137-85-6
MW (no tag):	719.7 Da
Formula:	C33H36F3N5O10
Source:	Synthetic
Tag:	N/A
Stock Buffer:	Powder
Solubility:	Soluble in DMSO
Concentration:	N/A
Quality Assurance:	> 95% by HPLC
Description:	Ac-YVAD-AFC is a fluorogenic substrate of caspase-1/4. 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 strongly 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. Make a 25 mM substrate stock in DMSO: add 0.278 mL DMSO to 5 mg Ac-YVAD-AFC powder or 1.39 mL DMSO to 25 mg Ac-YVAD-AFC powder. 3. Make 2X Reaction Buffer: 40 mM Tris, pH 7.6 at 4 ^{0}C, 300 mM NaCl, 4 mM DTT. 4. Prepare 2X substrate (100 µM): add 20 µl substrate stock prepared in step 3 to 5 ml warmed (37 ^{0}C) 2X reaction buffer. Briefly vortex to dissolve. 5. Mix 50 µl apoptotic cell/tissule 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 5-120 min (users should optimize incubation time). 6. Record AFC fluorescence using a fluorometer or plate reader using excitation/emmission wavelengths at 400 nm/505 nm, respectively. Alternatively, AFC fluorescence can be recorded continuously when the substrate is mixed with samples to initiate reactions. 7. A control reaction can be included and the reading from a control reaction should be subtracted as the background signal. Appropriate control includes mixing non-apoptotic cell/tissue lysates + substrate or mixing apoptotic cell/tissue lysate + substrate + a caspase inhibitor (such as 25 µM Z-VAD-FMK).
References:	Poreba M., et al., Cold Spring Harb Perspect Bio. 2013, a008680.