Mca-KKVAPYPME-Dap(Dnp)-NH_2

Cat. # G1000, G1001

Also Known as: LFP

Cas#: N/A

MW (no tag): 1.53 KDa

Formula: $C_{70}H_{96}N_{16}O_{21}S$

Source: Synthetic

Stock Format: Powder

Solubility: Soluble in DMSO up to 20 mM

Quality Assurance: >97% purity by HPLC. Cleaved by active proteasomes

Description: Mca-KKVAPYPME-Dap(Dnp)–NH_2 is a fluorogenic peptide substrate of proteasomes, in which the Mca fluorescence is internally quenched by Dap(Dnp). This substrate can be cleaved by mammalian, yeast or archaea activated proteasomes. Upon cleavage, the Mca fluorescence can be measured at excitation/emission wavelengths of 320/405 nm using a plate reader or fluorometer. Due to its long sequence as a nanomer, latent 20S proteasome has very weak activity on hydrolysis of Mca-KKVAPYPME-Dap(Dnp)–NH2, but it can be cleaved by activated proteasomes, including the 26S proteasome.

Mca-KKVAPYPME-Dap(Dnp)–NH_2 can be used for 1) in vitro assaying 20S proteasome activation (gate opening) by assembling with a regulatory particle; and 2) determining the change of active proteasome activity in cells or tissues.

The working concentration is 10-20 µM.

Mca-KKVAPYPME-Dap(Dnp)–NH_2 can be cleaved by other enzymes in cell or tissue extracts. MG132-treated samples should be used to deduct non-proteasome-mediated cleavage.

Storage: Eligible for room temperature shipping. Store at -20°C or -80°C upon receiving. Avoid multiple freeze-thaw cycles after dissolving in DMSO. Protect from light.

Data

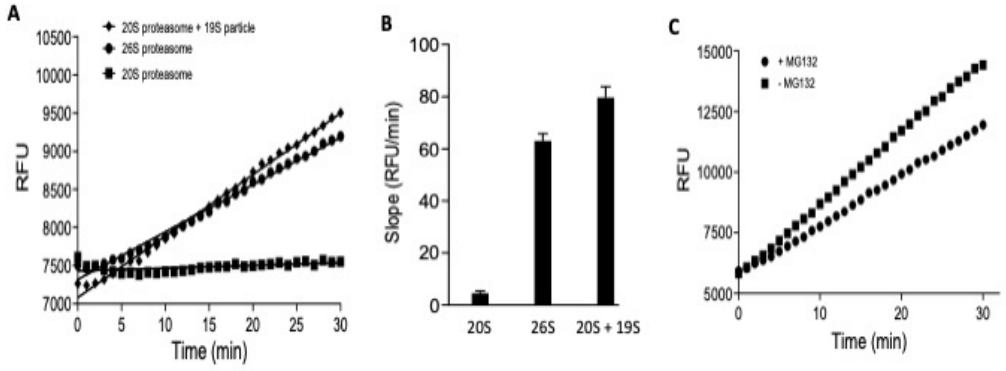

Figure Legend:

A. Determination of cleavage of Mca-KKVAPYPME-Dap(Dnp)–NH_2 by highly purified 4 nM bovine 20S proteasome, 4 nM 26S proteasome, or 4 nM 20S proteasome + 32 nM PA700 (see the protocol below).

B. Activity of 20S proteasome, 26S proteasome, or in vitro assemble 26S proteasome (20S + 19S) was calculated by the slope value of each curve in A, plotted as a bar graph.

C. 5 μg HEK293 cell lysates were incubated with DMSO or 100 μM MG132 at 37 ^{0}C for 10 min, then mixed with 20 μM Mca-KKVAPYPME-Dap(Dnp)–NH_2. In all assays, Mca-KKVAPYPME-Dap(Dnp)–NH_2 final concentration is 20 μM. Mca fluorescence was measured with a plate reader with excition and emmision wavelengths at 340 nm and 405 nm, respectively.

Protocol:

Activation of 20S proteasome by PA700 monitored by a fluorometric assay.

Step 1. Incubate 8 nM constitutive 20S proteasome (catalog # A1400) or 8 nM constitutive 20S proteasome + 64 nM PA700 (catalog # A1300) in 20 mM Tris, pH 7.1 at 37 ^{0}C, 20 mM NaCl, 2 mM βME, 5 mM $MgCl_2$, 2 mM ATP and 10% glycerol for 30 min at 37 ^{0}C in a water bath. A total of 100 μl for each sample.

Step 2. Add 2 μl Mca-KKVAPYPME-Dap(Dnp)-NH_2 stock (20 mM in DMSO) into 998 μl warmed buffer (20 mM Tris, pH 7.1 at 37 ^{0}C, 2 mM βME), vortex vigorously for 20 seconds, and keep at 37 ^{0}C.

Step 3. Add 25 μl each of the proteasome samples prepared in step 1 into wells a 96-well clear bottom black plate, then mixed with 25 μl substrate prepared in step 2. The Mca fluorescence was recorded immediately in a 30 min kinetic course using the exciting/emission wavelengths of 320/405 nm, respectively, using a BioTek plate reader. Each reaction was set up as triplicates.

References:

1.Smith D.M., et al., Mol. Cell, 2007, 27, 731.