

Product Name:	Angiotensin I Converting Enzyme 2, (ACE-2) Substrate	
Catalog Number:	AS-60757 (1 mg)	Lot Number: See label on vial
Sequence:	Mca-Ala-Pro-Lys(Dnp)-OH (3-letter code) Mca-APK(Dnp) (1-letter code)	
Molecular Weight:	696.7	
% Peak Area by HPLC: ≥ 95		

Appearance: Lyophilized yellow powder

Peptide Reconstitution: Reconstitute by adding 80-100 µl 1%NH₄OH to 1 mg ACE-2 substrate. Dilute this peptide solution to approximately 1 mg/ml (or more dilute) with a buffer such as PBS or another buffer; aliquot and store at -20C.

Storage: ACE-2 peptide is shipped at ambient temperature. Upon receipt, store lyophilized peptide at –20°C or lower. Reconstituted peptide can be aliquoted and stored at –20°C or lower.

Description: An ACE-2 (Angiotensin I-converting enzyme 2) fluorescent substrate. Complete hydrolysis of 0.04 mM results in a 300-fold fluorescence increase over background. Max Abs/Em=325/393 nm upon cleavage of substrate. Ref: Vickers, C. et al. *J. Biol. Chem.* **277**, 14838 (2002).

Additional Information: Listed below are relevant information that may provide a guideline on how to use this product. End users will have to adapt to their own specific applications.

The peptide Mca-APK(Dnp) was synthesized by Anaspec, Inc. (San Diego, CA). Mca-APK(Dnp) was dissolved in 100% Me2SO and quantitated by measuring absorbance at 350 nm using an extinction coefficient of 15,000 M⁻¹ cm⁻¹-<u>Vickers, C. et al. J. Biol. Chem. 277, 14838</u> (2002).

The ACE2 enzymatic assay was conducted using assay buffer (0.05M MES, 0.3M NaCl, 10MM ZnCl2; pH 6.8) and the synthetic substrate MCA-Ala-Pro-Lys-Dnp (AnaSpec, San Jose, CA). Twenty-µg protein was incubated with ACE2 assay buffer containing inhibitors (10MM lisinopril, 10µM SCH, 2µM amastatin, 10µM bestatin, 10µM benzyl succinate), and 1mM of substrate at 37°C for 1h-Joyner, J. et al. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 0: 00387.2006v1 (2007).

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