

PEPTIDE NAME: Mca-RPPGFSAFK(Dnp), Fluorogenic Substrate

SEQUENCE: Mca-Arg-Pro-Pro-Gly-Phe-Ser-Ala-Phe-Lys(Dnp)-OH [Mca=(7-methoxycoumarin-4-yl)acetyl; Dnp=2,4-dinitrophenyl]

SEQUENCE DESCRIPTION: Fluorogenic substrate for ECE-1 [k_{cat}/K_m ($M^{-1}s^{-1}$) 1.9×10^7]^{1,2} and ECE-2 (k_{cat}/K_m 7.9×10^5)², ACE and ACE2³, neprilysin (k_{cat}/K_m 1.7×10^6)¹, MMP-2 and MMP-9 ($k_{cat}/K_m \sim 1.5 \times 10^4$)¹, thimet oligopeptidase (TOP) (k_{cat}/K_m 5.3×10^6)⁴, as well as cathepsin A, cathepsin X/Z, BACE, and insulinase, but not MMP-1¹. Mca fluorescence is quenched by the Dnp group until cleavage separates them (ECE-1 cleaves the Ala-Phe bond). Ex.: 328nm, Em.: 393nm, but it is possible to use Ex./Em. 320-340/393-420nm. This substrate is useful for inhibitor screening and kinetic analysis.

USAGE: Dissolve the peptide with DMSO for a 10 mM stock solution. To ensure accurate concentration is achieved, both peptide purity and content need to be taken into account. Here is an example calculating the amount of DMSO needed to dissolve 1 mg Cat. # P-227 to 10mM, when its purity is 98% and content is 76.5%: $(\text{mol}/1388.5\text{g}) \times (1 \times 10^{-3} \text{ mmol/mol}) \times (L/10 \text{ mmol}) \times (1 \times 10^6 \text{ } \mu\text{l/L}) \times (\text{g}/1 \times 10^3 \text{ mg}) \times (0.765 \times 1 \text{ mg}) \times (0.98) = 54.0 \text{ } \mu\text{l}$ DMSO. Store at -20°C in aliquots.

CATALOG NO.: P-227

LOT NO.: temp

MOLECULAR WEIGHT: 1388.5 analysis.

PURITY: >98.6% by HPLC

AMINO ACID ANALYSIS AND IDENTITY: Confirms.

SUPPLIED AS: 1 mg gross peptide/vial.

PEPTIDE CONTENT: 75%

PHYSICAL APPEARANCE: Lyophilized solid.

SOLUBILITY: DMSO 10 mM

STORAGE: -20°C desiccated. Protect from light.

LITERATURE REFERENCES:

1. G.D. Johnson and K. Ahn *Anal. Biochem.* 2000 **286** 112
2. N. Mzhavia et al. *J. Biol. Chem.* 2003 278 14704
3. M.P. Ocaranza et al. *Revista Chilena de Cardiología* 2005 **24** 83
4. J.A. Sigman et al. *Biochem. J.* 2005 **388** 255

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