**DESCRIPTION**

**Source**  
Mouse myeloma cell line, NS0-derived  
Gln18-Ser740, with a C-terminal 10-His tag  
Accession # Q9BYF1

**N-terminal Sequence Analysis**  
No results obtained: Gln18 predicted

**Structure / Form**  
Recombinant Human ACE-2 is prone to proteolytic cleavage at C-terminus. The predominant form of the purified protein lacks the His tag.

**Predicted Molecular Mass**  
85 kDa

**SPECIFICATIONS**

**SDS-PAGE**  
120 kDa, reducing conditions

**Activity**  
Measured by its ability to cleave a fluorogenic peptide substrate, Mca-YVADAPK(Dnp)-OH (Catalog # ES007).  
The specific activity is >800 pmol/min/µg, as measured under the described conditions.

**Endotoxin Level**  
<1.0 EU per 1 µg of the protein by the LAL method.

**Purity**  
>90%, by SDS-PAGE under reducing conditions and visualized by silver stain.

**Formulation**  
Supplied as a 0.2 µm filtered solution in Tris, NaCl, ZnCl₂ and Glycerol. See Certificate of Analysis for details.

**Activity Assay Protocol**

**Materials**

- Assay Buffer: 75 mM Tris, 1 M NaCl, pH 7.5  
- Recombinant Human ACE-2 (rhACE-2) (Catalog # 933-ZN)  
- Substrate: MCA-Tyr-Val-Ala-Asp-Ala-Pro-Lys(DNP)-OH (Catalog # ES007), 10 mM stock in DMSO  
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)  
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

**Assay**

1. Dilute rhACE-2 to 0.2 ng/µL in Assay Buffer.  
2. Dilute Substrate to 40 µM in Assay Buffer.  
3. Load in a black well plate 50 µL of 0.2 ng/µL rhACE-2, and start the reaction by adding 50 µL of 40 µM Substrate. As a control load 50 µL of 40 µM Substrate with 50 µL of Assay Buffer.  
4. Read at excitation and emission wavelengths of 320 nm and 405 nm (top read), respectively in kinetic mode for 5 minutes.  
5. Calculate specific activity:

   \[
   \text{Specific Activity (pmol/min/µg) = } \frac{\text{Adjusted } V_{\text{max}} \times (\text{RFU/min}) \times \text{Conversion Factor} \times (\text{pmol/RFU})}{\text{amount of enzyme (µg)}}
   \]

   *Adjusted for Substrate Blank  
**Derived using calibration standard MCA-Pro-Leu-OH (Bachem, Catalog # M-1975)

**Final Assay Conditions**

**Per Well:**  
- rhACE-2: 0.010 µg  
- Substrate: 20 µM

**PREPARATION AND STORAGE**

**Shipping**  
The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.

**Stability & Storage**  
Use a manual defrost freezer and avoid repeated freeze-thaw cycles.  
- 6 months from date of receipt, -20 to -70 °C as supplied.  
- 3 months, -20 to -70 °C under sterile conditions after opening.

**BACKGROUND**

**ACE-2**, also called ACEH (ACE homolog), is an integral membrane protein and a zinc metalloprotease of the ACE family that also includes somatic and germinal ACE (1). Human ACE-2 has about 40% amino acid identity to the N- and C-terminal domains of human somatic ACE. The predicted human ACE-2 protein sequence consists of 805 amino acids, including a N-terminal signal peptide, a single catalytic domain, a C-terminal membrane anchor, and a short cytoplasmic tail. ACE-2 cleaves angiotensins I and II as a carboxypeptidase. ACE-2 mRNA is found at high levels in testis, kidney and heart and at moderate levels in colon, small intestine and ovary. Classical ACE inhibitors such as captopril and lisinopril do not inhibit ACE-2 activity. Novel peptide inhibitors of ACE-2 do not inhibit ACE activity (2). Genetic data from *Drosophila*, mice and rats show that ACE-2 is an essential regulator of heart function in vivo (3).

**References:**