DESCRIPTION

Source
Mouse myeloma cell line, NS0-derived human Proprotein Convertase 1/PCSK1 protein Ser111-Arg617, with a C-terminal His tag
Accession # P29120

N-terminal Sequence Analysis
Ser111

Structure / Form
Mature form

Predicted Molecular Mass
57 kDa

SPECIFICATIONS

SDS-PAGE
63-80 kDa, reducing conditions

Activity
Measured by its ability to cleave the fluorogenic peptide substrate pERTKR-AMC (Catalog # ES013). The specific activity is >30 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
>95%, by SDS-PAGE under reducing conditions and visualized by silver stain.

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

Activity Assay Protocol

Materials
- Assay Buffer: 25 mM MES, 5 mM CaCl₂, 1% (w/v) Brij-35, pH 6.0.
- Recombinant Human Proprotein Convertase 1/PCSK1 (rhPCSK1) (Catalog # 2810-SE)
- Substrate: L-PyroGlu-Arg-Thr-Lys-Arg-AMC (pERTKR-AMC) (Catalog # ES013)
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay
1. Dilute rhPCSK1 to 4 µg/mL in Assay Buffer.
2. Dilute Substrate to 200 µM in Assay Buffer.
3. Load 50 µL of 4 µg/mL of rhPCSK1 into a plate, and start the reaction by adding 50 µL of 200 µM Substrate. Include a Substrate Blank containing 50 µL of Assay Buffer and 50 µL of Substrate.
4. Incubate at 37 °C for 1 hour.
5. Read at excitation and emission wavelengths of 380 nm and 460 nm (top read), respectively in endpoint mode.
6. Calculate specific activity:

\[
\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted Fluorescence}^* \times \text{Conversion Factor}^**}{\text{Incubation time (min) x amount of enzyme (µg)}}
\]

*Adjusted for Substrate Blank
**Derived using calibration standard 7-Amino, 4-Methyl Coumarin (AMC) (Sigma, Catalog # A9891).

Final Assay Conditions Per Well:
- rhPCSK1: 0.200 µg
- Substrate: 100 µM

PREPARATION AND STORAGE

Shipping
The product is shipped with dry ice or equivalent. 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, -70 °C as supplied.
- 3 months, -70 °C under sterile conditions after opening.

BACKGROUND

The human PCSK1 gene encodes Proprotein Convertase 1 (PC1), which is also known as PC3 (1, 2). As a serine protease of the furin/PC family, the deduced amino acid sequence of human PC1 consists of a signal peptide (residues 1 to 27), a pro peptide (residue 28 to 110), and a mature chain (residues 111 to 753). Autocatalytic processing results in the removal of the pro peptide as well as a region from the C-terminus, which leads to the production of the most active 66 kDa form (3). The purified rhPC1 consists of residues 111 to 617, is active in the assay described above and most likely corresponds to the 66 kDa form. Found almost exclusively in endocrine and neural cells and stored as the 66 kDa form in dense-core secretory vesicles, PC1 is capable of processing several endocrine and neural prohormones, such as opiomelanocortin, somatostatin, enkephalin, insulin, dynorphin and thyrotropin-releasing hormone (3). Indeed, obesity and impaired prohormone processing have been associated with mutations in the human PCSK1 gene (4).

References: