

## Instruction Manual

<b>Catalog Number</b>	PK-RP577-1081-25
<b>Description</b>	Caspase-1 (also known as ICE) is a prototypical member of the caspase-family of cysteine proteases. Caspase-1 exists in cells as an inactive 45 kDa proenzyme. The pro-enzyme is matured by proteolysis to yield large (20 kD) and small (10 kD) subunits. The active caspase-1 is a heterotetramer consisting of two large and two small subunits. To date the regulatory mechanism of caspase-1 activation and the role of caspase-1 in apoptosis are poorly understood. In THP-1 cells, a large proportion of the caspase-1 is present in the inactive proenzyme form. The recombinant active human caspase-1 was expressed in E. coli. The active caspase-1 preferentially cleaves caspase-1 substrates (e.g., YVAD-AFC or YVAD-pNA) and is routinely tested for its ability to enzymatically cleave these two substrates Ac-YVAD-pNA or Ac-YVAD-AFC. The rHu Caspase has an N-terminal His-tag and corresponds to amino acids 120-404 of Caspase-1, gene accession# NP_150634.1.
<b>Quantity</b>	25 units
<b>Specific Activity</b>	>5,000 units/mg
<b>Unit Definition</b>	One unit of the recombinant caspase-1 is the enzyme activity that cleaves 1 nmol of the caspase substrate YVAD-pNA (pNA: p-nitroanaline) per hour at 37°C in a reaction solution containing 50 mM Hepes, pH 7.2, 50 mM NaCl, 0.1% Chaps, 10 mM EDTA, 5% Glycerol, and 10 mM DTT.
<b>Formulation</b>	Lyophilized or semi-dry powder
<b>Reconstitution</b>	The active recombinant caspases can be reconstituted to 0.1-1 unit per µl in PBS or - for longer stability - in PBS containing 15% glycerol or the Reaction Buffer described above (also available separately from PromoKine, Cat. No. PK-CA577-1068-20 and PK-CA577-1068-80). We recommend using 1 unit per assay for analyzing caspase activity.
<b>Storage</b>	The lyophilized caspase-1 is stable for 1 year at -70°C. Following reconstitution in PBS + 15% glycerol, the enzyme should be aliquoted and immediately stored at -70°C. Avoid multiple freeze/thaw cycles as activity might decrease.
<b>Applications</b>	Active caspase-1 is useful in studying enzyme regulation, determining target substrates, screening caspase inhibitors, or as a positive control in caspase activity assays. We recommend using 1 unit/assay for analyzing caspase activity. For a complete caspase-1 assay protocol, please refer to PromoKine's Caspase-1 Fluorometric or Colorimetric Assay Kits.

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