

Instruction Manual

Catalog Number	PK-RP577-1083-25
Description	Caspase-3 (also known as CPP32, Yama and apopain) is a major member of the caspase-family of cysteine proteases. Caspase-3 exists in cells as an inactive 32 kDa proenzyme. During apoptosis procaspase-3 is processed at aspartate residues by self-proteolysis and/or cleavage by upstream caspases, such as caspase-6 (Mch2), caspase-8 (Flice) and granzyme B. The processed form of caspase-3 consists of large (17 kD) and small (11 kD) subunits which associate to form the active enzyme. The active caspase-3 has been shown involving in the proteolysis of several important molecules, such as poly (ADP-ribose) polymerase (PARP), the sterol regulatory element binding proteins (SREBPs), focal adhesion kinase (FAK), and others. The recombinant active human caspase-3 expressed in E. coli spontaneously undergoes autoprocessing to yield subunits characteristic of the native enzyme. The active caspase-3 preferentially cleaves caspase-3 substrates (e.g., DEVD-AFC or DEVD-pNA) and is routinely tested for its ability to enzymatically cleave these two substrates Ac-DEVD-pNA or Ac-DEVD-AFC.
Quantity	25 units
Specific Activity	>15,000 units/mg
Unit Definition	One unit of the recombinant caspase-3 is the enzyme activity that cleaves 1 nmol of the caspase substrate DEVD-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.
Formulation	Freeze-dried powder with additives.
Reconstitution	The active recombinant caspases can be reconstituted to 1 unit per µl in water 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.
Storage	The lyophilized caspase-3 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.
Applications	Active caspase-3 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-3 assay protocol, please refer to PromoKine's Caspase-3/ CPP32 Fluorometric or Colorimetric Assay Kits.

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