Active Caspase 3 (CASP3) Instruction Manual

SBPA154Hu01

Homo sapiens (Human)

Buffer Formulation	PBS, pH7.4, containing 0.01% SKL, 5% Trehalose.
Traits	Freeze-dried powder
Purity	> 90%
Isoelectric Point	7.1
Applications	Cell culture; Activity Assays.

ACTIVITY TEST

Calculation

$$\text{Activity, pmol pNA/min/μg} = \frac{OD \times d}{\varepsilon^{mM} \times t} \text{ /amount of protein}$$

Where: $\varepsilon^{mM} = 10.5$

d - dilution factor

t - reaction time in minutes

Caspase 3 is a member of the cysteine-aspartic acid protease (caspase) family. Sequential activation of caspases plays a central role in the execution-phase of cell apoptosis. Caspases exist as inactive proenzymes that undergo proteolytic processing at conserved aspartic residues to produce two subunits, large and small, that dimerize to form the active enzyme. This protein cleaves and activates caspases 6 and 7; and the protein itself is processed and activated by caspases 8, 9, and 10. Caspase 3 can hydrolyze the peptide substrate acetyl-Asp-Glu-Val-Asp-p-nitroanilide (Ac-DEVD-pNA) resulting in the release of the p-nitroaniline (pNA) moiety. p-Nitroaniline has a high absorbance at 405nm. Thus the activity of recombinant human caspase 3 can be measured by calculate the concentration of the pNA released from the substrate. The reaction was performed in adding 50μL 2×buffer (50mM HEPES, 100mM NaCl, 10mM DTT, 2mM EDTA, 10% glycerol) to 96 well plates, then add 50μL various concentration of caspe 3 (diluted by 1×buffer, 25mM HEPES, 50mM NaCl, 5mM DTT, 1mM EDTA, 5% glycerol) to each well, finally, add 5μL 4mmol Ac-DEVD-pNA to each well. Cover the 96 well plates and

incubate at 37℃ for 2h. p-Nitroaniline (pNA) standard curve prepare by double dilute 200μM pNA with 1×buffer and record the OD value at 405nm. Calculate the caspase 3 activity in pmol of pNA released per min per μg recombinat human caspase 3.
The specific activity of recombinant human caspase 3 is 2196pmol/min/μg.

USAGE

Reconstitute in ddH_2O to a concentration ≤0.1mg/mL. Do not vortex.

STORAGE

Avoid repeated freeze/thaw cycles. Store at 2-8°C for one month. Aliquot and store at -80°C for 12 months.

STABILITY

The thermal stability is described by the loss rate. The loss rate was determined by accelerated thermal degradation test, that is, incubate the protein at 37°C for 48h, and no obvious degradation and precipitation were observed. The loss rate is less than 5% within the expiration date under appropriate storage condition.

Image

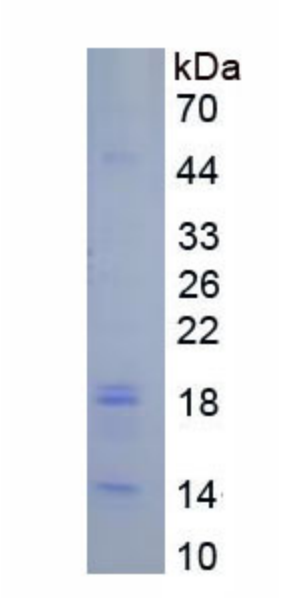

Figure. SDS-PAGE

[IMPORTANT NOTE]

The kit is designed for research use only, we will not be responsible for any issue if the kit was used in clinical diagnostic or any other procedures.