

Product Information

p35 Protein from baculovirus, recombinant

C-terminal histidine tagged
expressed in *E. coli*

Product Number **P 9361**
Storage Temperature $-20\text{ }^{\circ}\text{C}$

Product Description

This product is recombinant p35 protein from baculovirus containing a C-terminal His-tag expressed in *E. coli*. It is purified by metal-chelate chromatography.¹

The anti-apoptotic protein p35 from baculovirus is thought to prevent the suicidal response of infected insect cells by inhibiting multiple forms of caspases. p35 protein inhibits human caspases 1, 3, 6, 7, and 10, with K_i values ranging from 0.1 to 9 nM.² This inhibition is stoichiometric with a ratio of approximately 1.0 between the p35 protein and the caspase. p35 protein has been shown to be a specific inhibitor of the caspases with virtually no inhibition of other serine or cysteine proteases, such as Granzymes A, B, and K, trypsin, chymotrypsin, papain, or subtilisin.

The product is supplied as a solution in 50 mM HEPES, pH 7.6, containing 1 mM DTT, 150 mM NaCl, 0.1% CHAPS and 50% glycerol.

Activity: minimum 5,000 units per mg protein

Unit Definition: One unit will cause the inhibition of 50% of the activity of 200 ng of caspase-3 (Product No. C 1224) assayed on Ac-DEVD-pNA (Product No. A 2559) at pH 7.4 at 37 °C.

Purity: minimum 90% (SDS-PAGE)

Precautions and Disclaimer

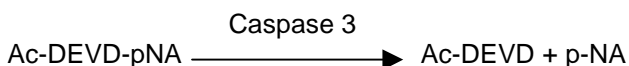
This product is for laboratory research use only. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The product ships on dry ice and it is recommended to store the product at $-20\text{ }^{\circ}\text{C}$. The product is stable for 2 years when stored properly.

Procedure

The assay of p35 protein from baculovirus is based on the inhibition of caspase activity. Caspase 3 is the substrate of choice, as it has been reported to be the caspase most sensitive to this inhibitor with a K_i of $1.1 \times 10^{-10}\text{ M}$.² Caspase activity is determined using the colorimetric substrate Ac-DEVD-pNA with the absorbance of the released p-nitroaniline (p-NA) measured at 405 nm.



The p35 protein inhibitor must be allowed to bind to caspase 3 for inhibition; therefore, a preincubation of 20 minutes should be performed prior to assaying for enzyme activity. Longer times up to 30 minutes are acceptable, but not necessary. Incubation for less than 10 minutes will result in incomplete binding and inhibition by the p35 protein.

For inhibition by endogenous samples, a titration of the inhibitor will be necessary. Total inhibition of caspase 3 activity occurs with 3 to 4 times the amount needed to cause 50% inhibition.

Reagents required for the assay procedure

- Reaction Buffer: 25 mM HEPES, pH 7.4, containing 2 mM EDTA, 0.1% CHAPS, and 5 mM DTT
- Caspase 3 Substrate Solution – colorimetric substrate Ac-DEVD-pNA (Product No. A 2559): Dissolve the substrate in dry DMSO (Product No. D 8418) at 12.75 mg/ml (20 mM).
- Caspase 3 Solution (Product No. C 1224) - Dilute the enzyme with assay buffer to bring the protein concentration to 20 $\mu\text{g/ml}$. Use 200 ng of enzyme (10 μl) per reaction mixture.
- p35 Protein Inhibitor Solution - Solution diluted to 8-12 $\mu\text{g/ml}$ with assay buffer.
- Stop solution: 5 M NaOH

Reaction Scheme

Sample	Caspase 3 Solution	p35 Protein Inhibitor Solution	Reaction Buffer	Caspase 3 Substrate Solution
Control	0	0	495 μ l	5 μ l
Caspase 3 Reaction	10 μ l	0	485 μ l	5 μ l
Inhibition of Caspase 3	10 μ l	x μ l	(485-x) μ l	5 μ l

At least 3 volumes of each inhibitor sample should be assayed in order to find the 50% inhibition point (use 3 volumes to give a range of 25 to 80 ng of p35 protein). The absorbance is measured with a spectrophotometer at 405 nm using a 0.5 ml glass or quartz cuvette. The Reaction Buffer should be maintained at a temperature of 37 °C.

1. Place 10 μ l of the Caspase 3 Solution in a 1.5 ml microcentrifuge tube.
2. Add the p35 Protein Inhibitor Solution, bring the volume to 495 μ l with Reaction Buffer, and vortex.
3. Incubate at 37 °C for 20 minutes.
4. Start the reaction by addition of 5 μ l of Caspase 3 Substrate Solution and vortex.
5. After 10 minutes stop the reaction by addition of 2.5 μ l of the Stop Solution (5 M NaOH) and vortex.
6. Use the Reaction Buffer alone in the reference cuvette of the spectrophotometer or blank the instrument using Reaction Buffer alone.
7. Measure and record the absorbance of the Control reaction.
8. Measure and record the absorbance of the reaction tubes.

Calculation

1. From each sample subtract the value for the Control reaction without enzyme.
2. Using the value obtained for the Caspase 3 Reaction (enzyme without p35 protein present) as 100%, calculate the percent of activity in each of the samples containing the p35 Protein Inhibitor Solution.
3. Plot the percent of activity against ng of p35 protein inhibitor present and determine from the graph the amount of p35 protein that will result in 50% inhibition of the caspase 3 activity.

References

1. Bertin, J. et al., J. Virol., **70**, 6251 (1996).
2. Zhou, Q. et al., Biochem., **37**, 10757 (1998).

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