

Background

Caspase-3 is a critical enzyme involved in the process of apoptosis, or programmed cell death. It's considered one of the "effector caspases," which are the final executioners of the apoptotic process. Caspase-3 is activated in response to various cellular stress signals and, once activated, it leads to the breakdown of cellular components and the death of the cell. This ensures that damaged or unwanted cells are efficiently removed without causing harm to the surrounding tissue. In the context of cancer, the role of caspase-3 is significant because cancer cells often evade apoptosis, allowing them to survive and proliferate uncontrollably. The dysregulation of apoptosis is a hallmark of cancer, and as a result, many cancer cells have reduced or altered caspase-3 activity. Because of its essential role in apoptosis, caspase-3 has been studied in the context of cancer (where apoptosis may be disrupted) and neurodegenerative diseases (where increased apoptosis can occur).

Assay Principle

Caspase-3 activity assay kit is designed to measure caspase-3 activity for enzyme profiling and inhibitor screening. Proteolytic activity of caspase-3 cuts the fluorogenic substrate and releases the fluorophore, resulting in fluorescent intensity increase which can be measured using a microplate reader at excitation at 360 nm and emission at 460 nm.

Application

Quantification of caspase-3 activity and High throughput screening of compounds that have effects on the enzyme activity for drug discovery.

Plate Reader

A microplate reader capable of measuring fluorescence intensity at excitation at 360 nm and emission at 460 nm.

Components

Catalog number	Item	Amount	Storage
810033	2x Caspase assay buffer	25 mL	-20°C
810031	Recombinant human caspase-3	7 µL	-80°C
810032	1 mM Caspase-3 substrate	80 µL	-80°C
	384-well microplate, White	1	Room temperature

Materials needed but not supplied

1. Microplate reader
2. 0.5 M DTT
3. Adjustable micro-pipettor
4. Sterile Tips

Assay protocol

1. Prepare assay buffer containing 1 mM DTT (1X DTT-containing assay buffer)

For example, mix 998 μ l distilled water with 1000 μ l of 2X assay buffer (Catalogue number: 810033) and 4 μ l of 0.5 M DTT. Make only enough DTT-containing assay buffer as needed for the assay. Store the remaining assay buffer at -20°C .

2. Prepare the inhibitor compound solution

If the inhibitor compound is dissolved in water, make a solution of the compound 10-fold higher than the final concentration in 1X assay buffer (since you will add 2 μ l to the 20 μ l reaction).

If the inhibitor compound is dissolved in DMSO, make a 100-fold higher concentration of the compound than the highest concentration you want to test in DMSO. Then make a 10-fold dilution in 1X assay buffer (at this step, the compound concentration is 10-fold higher than the final concentration and the DMSO concentration is 10%). To determine an IC_{50} or to test lower concentrations of the compound, prepare a series of further dilutions in 1X assay buffer containing 10% DMSO (the final concentration of the DMSO will be 1% in all samples).

3. Prepare caspase-3 solution

Thaw caspase-3 protein on ice. Upon first thaw, briefly spin tube to recover the full contents at the bottom of the tube. Make aliquots of the enzyme for single use. Store remaining undiluted protein at -80°C .

Note: caspase-3 protein is sensitive to freeze/thaw cycles. Limit the number freeze-thaw cycles for best results. Do not re-use the diluted protein.

Dilute the caspase-3 protein 500-fold (1 μ l caspase-3 + 499 μ l 1X DTT-containing assay buffer).

Add 8 μ l of diluted protein solution to each positive control wells and inhibitor test wells.

Add 8 μ l of 1X DTT containing buffer to each of the negative control wells.

4. Add inhibitor solution

Add 2 μ l of diluted compound solution to each inhibitor test well.

Add 2 µl of inhibitor solvent solution to each negative and positive control wells.

Incubate at room temperature for 30 minutes (optional).

5. Prepare caspase-3 substrate

Thaw the substrate at room temperature.

Dilute the substrate 50-fold (1 µL of 1 mM tracer + 49 µL 1X DTT-containing assay buffer).

Add 10 µl of diluted substrate to each well.

Dilute enough substrate for single use. Store remaining undiluted tracer at -80°C. Do not re-use the diluted tracer.

6. Incubate the reaction at room temperature for 60 minutes.

7. Measure fluorescent intensity

Fluorescent intensity should be measured by excitation wavelength at 360 nm and emission at 460 nm.

Protocol Summary

Component	Negative Control	Positive Control	Inhibitor Test
1X buffer	8 µl		
Caspase-3		8 µl	8 µl
Inhibitor solvent	2 µl	2 µl	
Inhibitor solution			2 µl
Subtotal Volume	10 µl	10 µl	10 µl
Incubate at room temperature for 30 minutes (Optional).			
Caspase-3 substrate	10 µl	10 µl	10 µl
Total Volume	20 µl	20 µl	20 µl

Incubate at room temperature for 2 hours.

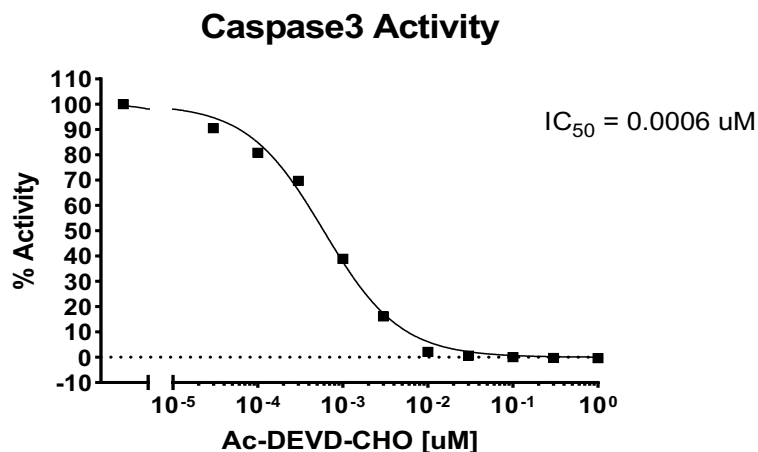
Data Analysis

1. Calculate percentage activity

In the absence of the compound (positive control), the sample signal (P) is defined as 100% activity. In the absence of enzyme (negative control), the sample signal (N) is defined as 0% activity. The percent activity in the presence of each compound is calculated according to the following equation: % activity = (S-N)/(P-N) X100, where S= the sample signal in the presence of the compound.

$$\% \text{ Displacement} = \left(1 - \frac{S - N}{P - N}\right) \times 100$$

Assay result



Related products:

Catalog #	Product Name	Size
5727-4121NK	Kras WT Nucleotide Exchange Assay Kit	384 reactions
5727-4122NK	Kras G12C Nucleotide Exchange Assay Kit	384 reactions
5727-4123NK	Kras G12D Nucleotide Exchange Assay Kit	384 reactions
5727-4133NK	Kras G13D Nucleotide Exchange Assay Kit	384 reactions
5727-4127NK	Kras G12R Nucleotide Exchange Assay Kit	384 reactions
5727-4128NK	Kras G12V Nucleotide Exchange Assay Kit	384 reactions
5727-4121BK	Kras WT-cRAF Binding Assay Kit	384 reactions
5727-4122BK	Kras G12C-cRAF Binding Assay Kit	384 reactions
5727-4123BK	Kras G12D-cRAF Binding Assay Kit	384 reactions
5727-4127BK	Kras G12R-cRAF Binding Assay Kit	384 reactions
5727-4128BK	Kras G12V-cRAF Binding Assay Kit	384 reactions
756981BK	PKMYT1 Binding Assay Kit	384 reactions
759331BK	WEE1 Binding Assay Kit	384 reactions
34343-BK	eIF4E/eIF4G Binding Assay Kit	384 reactions
362101	DNA Polymerase Theta Activity Assay Kit	96-384 reactions
K777627	T7 High Yield RNA Synthesis Kit	25-100 reactions
728203	SARS-CoV-2 Mpro (3CL Protease) Assay Kit	96 reactions
728253	Papain-like Protease Assay Kit	96 reactions
728263	SARS-CoV-2 Nucleocapsid Protein Binding Kit (For mouse antibody)	384 reactions
728273	SARS-CoV-2 Nucleocapsid Protein Binding Kit (For rabbit antibody)	384 reactions

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