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Data Sheet
Homogeneous Caspase-8 Assay Kit
Catalog #80704
Size: 96 reactions

DESCRIPTION: Caspase-8 is a member of the caspase (cysteine aspartate protease) family of proteins, and has been shown to be an executioner protein of apoptosis. Caspase-8 has been associated with chronic inflammation and neurodegenerative disorders including Alzheimer's, Huntington's, and Parkinson's. The *Homogeneous Caspase-8 Assay Kit* is a complete assay system designed to measure Caspase-8 activity for screening and profiling applications. It comes in a convenient 96-well format, with all the reagents necessary for 100 fluorescent Caspase-8 activity measurements. In addition, the kit includes purified Caspase-8 enzyme and a potent Caspase-8 inhibitor, Ac-IETD-CHO, for use as a positive and negative control. Using this kit, only one simple step on a microtiter plate is needed to analyze the Caspase-8 activity level. The fluorogenic substrate, Ac-IETD-AFC, is incubated with purified Caspase-8 and the enzymatic activity releases AFC fluorophore that can then be measured using a fluorescence reader.

COMPONENTS:

Catalog #	Component	Amount	Storage	
80114	Recombinant Human Caspase-8	>1 µg	-80°C	Avoid freeze/thaw cycles!
	Caspase-8 Substrate (0.1 mM)	50 µl	-80°C	
	5X Caspase Assay Buffer 1	20 ml	-20°C	
	DTT (0.5 M)	200 µl	-20°C	
	Ac-IETD-CHO (100 µM)	20 µl	-80°C	
	black, low binding NUNC black microtiter plate	1	Room Temperature	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:

Fluorescent microplate reader capable of reading exc/em=380nm/500nm

APPLICATIONS: Great for studying enzyme kinetics and HTS applications.

STABILITY: One year from date of receipt when stored as directed.

REFERENCES:

1. Siegmund, D., *et al.*, *Mol Cell Biol.* 2005 August; **25(15)**: 6363–6379.
2. Dickens L.S., *et al.*, *Mol Cell.* 2012 Jul 27;**47(2)**:291-305.

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ASSAY PROTOCOL:

All samples and controls should be tested in duplicate.

- 1) Prepare 1X assay buffer containing 5 mM DTT from 5X assay buffer. For example, add 200 μ l 5X assay buffer and 10 μ l 0.5M DTT to 790 μ l H₂O to make 1 ml 1X assay buffer.
- 2) Prepare the master mixture: N wells \times (24.5 μ l 1X assay buffer + 0.5 μ l 0.1 mM Caspase-8 substrate).
- 3) Add 25 μ l of master mixture to wells labeled "Positive Control", "Test Sample", "Negative Control", and "Blank".
- 4) Prepare solution of test inhibitor that is 10-fold higher than the final concentration.
- 5) Add 5 μ l of the test inhibitor solution to the wells designated as "Test Sample". Add 5 μ l of the inhibitor buffer (without inhibitor) to the wells labeled "Blank" and "Positive Control". Add 5 μ l of control compound, Ac-IETD-CHO, to the wells designated "Negative Control".
- 6) Thaw Caspase-8 on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Aliquot Caspase-8 into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C. *Note: Caspase-8 enzyme is sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*
- 7) Dilute Caspase-8 in 1x assay buffer at 0.25 ng/ μ l (5 ng per reaction).
- 8) Add 20 μ l of 1x assay buffer to wells designated "Blank".

Component	Positive Control	Test Sample	Negative Control	Blank
1X assay buffer	24.5 μ l	24.5 μ l	24.5 μ l	44.5 μ l
Substrate (0.1mM)	0.5 μ l	0.5 μ l	0.5 μ l	0.5 μ l
Test Inhibitor	-	5 μ l	-	-
Ac-IETD-CHO	-	-	5 μ l	-
Inhibitor Buffer (no inhibitor)	5 μ l	-	-	5 μ l
Caspase-8 (0.25 ng/ μ l)	20 μ l	20 μ l	20 μ l	-
Total	50 μl	50 μl	50 μl	50 μl

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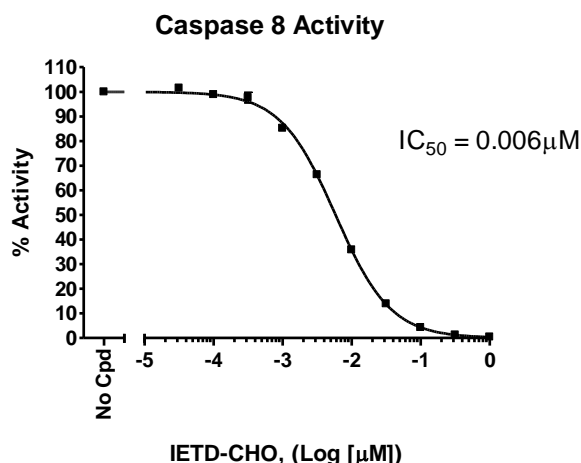
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- 9) Initiate reaction by adding 20 μ l diluted Caspase-8 solution to the wells labeled "Positive Control", "Test Sample" and Negative Control".
- 10) Incubate at room temperature for 30 minutes. Measure the fluorescence intensity in a microtiter plate-reading fluorimeter capable of excitation at 380 nm and detection of emission at 500 nm. "Blank" value is subtracted from all other values. Fluorescence measurements may be read over time to determine enzyme kinetics.

EXAMPLE OF ASSAY RESULTS:



Inhibition of Caspase-8 enzyme activity using Ac-IETD-CHO, measured using the *Homogeneous Caspase-8 Assay Kit*, BPS Bioscience #80704. Fluorescence intensity was measured using a Tecan fluorescent microplate reader. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com*

RELATED PRODUCTS

<u>Name</u>	<u>Catalog #</u>	<u>Size</u>
Caspase-3 Protein	80500	50 μ g
Caspase-6 Protein	80113	50 μ g
Caspase-7 Protein	70000	50 μ g
Caspase-8 Protein	80114	50 μ g
Caspase-3 Assay Kit	80700	96 rxns
Caspase-6 Assay Kit	80703	96 rxns
Caspase-7 Assay Kit	80701	96 rxns
XIAP (Bir2, Bir3)	75002	50 μ g
XIAP (Bir3)	75001	50 μ g

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