Description

Caspase-7 Homogeneous Assay Kit is a kit designed to measure caspase-7 activity for screening and profiling applications. The assay kit comes in a convenient 96-well format, with enough recombinant caspase-7, fluorogenic substrate, and assay buffer for 100 enzyme reactions. This kit also contains Ac-DNLD-CHO, a potent caspase-7 inhibitor, as control.

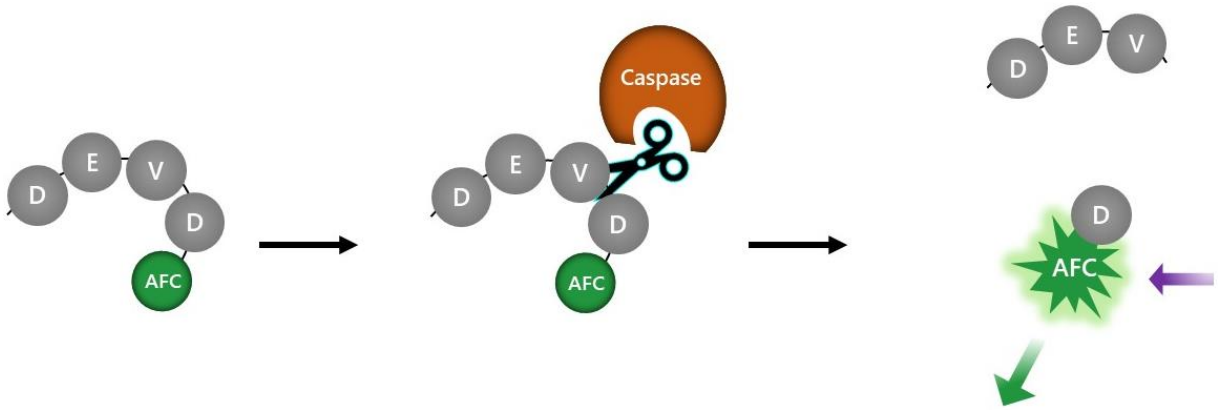

Figure 1: Illustration of the mechanism behind the Caspase-7 Homogeneous Assay Kit.
The substrate Ac-DEVD-AFC is incubated with caspase-7. Proteolysis of the substrate releases the AFC fluorophore. Fluorescence intensity can be measured with a microplate reader able to read fluorescence with λex=400 nm and λem = 505 nm. Fluorescence is thus proportional to caspase-7 activity.

Background

Caspase-7 is a cysteine-aspartic acid protease of the caspase family of proteins, belonging to group II (apoptotic executioner caspases). It plays a role in cell differentiation, tissue regeneration, apoptosis and neural development. It is synthesized as a zymogen, or procaspase, requiring cleavage by initiator caspases to trigger apoptosis. Its activity results in several cell changes, such as membrane blebbing, DNA fragmentation and the formation of apoptotic vesicles. Cell death via apoptosis can occur by intrinsic and extrinsic pathways. In response to DNA damage, metabolic or ER (endoplasmic reticulum) stress the intrinsic pathway can be activated. One example is the use of chemotherapeutic drugs. The release of cytochrome c from the mitochondria results in the formation of the APAF1 (apoptotic protease activating factor 1) apoptosome, recruitment of caspase-9 and finally conversion of procaspase-7 to caspase-7. Cells keep a tight control of caspase activity and express a number of inhibitors such as IAP (inhibitor of apoptosis) proteins. Caspase-7 can be inhibited by XIAP (X-linked IAP), cIAP1 and cIAP2. Deregulation of caspase activity can result in cancer, as many of the regulators of their activity are oncogenes or tumor suppressors. Caspase-7 has been used in the treatment of cancer, osteoarthritis, heart failure and neurogenerative disorders. The use of caspase-3/7 non-specific inhibitor have proved beneficial in maintaining cartilage homeostasis and potentially preventing and treating osteoarthritis. Further understanding of the role of caspase-7 in disease will allow us to develop innovative and efficacious new therapies.

Applications

Study enzyme kinetics and screen small molecule inhibitors for drug discovery and high throughput screening (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
70000	Caspase-7, His-Tag*	5 µg	-80°C
83524	0.1 mM Caspase-7 Substrate	50 µl	-80°C
82989	5x Caspase Assay Buffer 1	20 ml	-20°C
82735	0.5 M DTT	200 µl	-20°C
82747	100 µM Ac-DNLD-CHO	20 µl	-80°C
79685	Black, low binding microtiter plate	1	Room Temperature

**The concentration of the protein is lot-specific and will be indicated on the tube.*

Materials Required but Not Supplied

- Fluorimeter capable of excitation at λ=400 nm and detection at λ=505 nm
- Adjustable micropipettor and sterile tips
- Orbital shaker

Storage Conditions

This assay kit will perform optimally for up to **6 months** from date of receipt when the materials are stored as directed.

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Contraindications

- The final concentration of DMSO in the assay should not exceed 1%.
- Compounds that are fluorescent may interfere with the results, depending on their spectral excitation and emission properties.
- It is recommended that the compound alone be tested to determine any potential interference of the compound with the assay results.

Assay Protocol

- All samples should be run in duplicate while controls should be performed in quadruplicate.
- The assay should include “Blank”, “Positive Control”, “Control Inhibitor” and “Test Inhibitor” conditions.
- We recommend maintaining the diluted protein on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).
- We recommend using Ac-DNLD-CHO as internal control. If not running a dose response curve for the control inhibitor, we recommend running the control inhibitor at 0.1X, 1X and 10X the IC_{50} value shown in the validation data below.

1. Thaw **5x Caspase Assay Buffer 1**, **0.5 M DTT** and **0.1 mM Caspase-7 Substrate**.

2. Prepare 1x Caspase-7 Assay Buffer with 5 mM DTT by diluting together 5-fold the 5x Caspase Assay Buffer 1 and 100-fold of 0.5 M DTT with distilled water.

   Note: For example, to make 1 ml of 1x Caspase-7 Assay Buffer add 200 µl of 5x Capase-3 Assay Buffer 1, 10 µl of 0.5 M DTT and 790 µl of distilled water.

3. Prepare a **Master Mix** (25 µl/well): N wells x (24.5 µl of 1x Caspase-7 Assay Buffer + 0.5 µl of 0.1 mM Caspase-7 Substrate).

4. Add 25 µl of Master Mix to every well.

5. Prepare the **Test Inhibitor** (5 µl/well): for a titration, prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final volume of the reaction is 50 µl.

   5.1 If the Test Inhibitor is water-soluble, prepare 10-fold more concentrated serial dilutions of the inhibitor than the desired final concentrations in 1x Caspase-7 Assay Buffer.

   For the positive and negative controls, use 1x Caspase-7 Assay Buffer (Diluent Solution).

   OR

   5.2 If the Test inhibitor is soluble in DMSO, prepare the test inhibitor at a concentration 100-fold higher than the highest desired concentration in 100% DMSO, then dilute the inhibitor 10-fold in 1x Caspase-7 Assay Buffer to prepare the highest concentration of the 10-fold intermediate dilutions. The concentration of DMSO is now 10%.

   Using 1x Caspase-7 Assay Buffer containing 10% DMSO to keep the concentration of DMSO constant, prepare serial dilutions of the Test Inhibitor at 10-fold the desired final concentrations.

   For positive and negative controls, prepare 10% DMSO in 1x Caspase-7 Assay Buffer (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

   Note: The final concentration of DMSO should not exceed 1%.

6. Add 5 µl of Test Inhibitor to each well labeled “Test Inhibitor”.

7. Add 5 µl of Diluent Solution to the “Positive Control" and “Blank” wells.

8. Prepare the Inhibitor Control by diluting **100 µM Ac-DNLD-CHO** to 1000X the IC_{∞} in 100% DMSO. Then dilute 10-fold in 1x Caspase-7 Assay Buffer (the DMSO amount is now 10%) and corresponds to 100X the IC_{∞} value (5 µl/well). Using Diluent Solution prepare solutions at 1X and 10X the IC_{50} value (5 µl/well).

9. Add 5 µl of diluted Ac-DNLD-CHO to the “Control Inhibitor” wells.

10. Thaw **caspase-7** on ice. Briefly spin the tube containing the enzyme to recover its full content.

11. Dilute caspase-7 enzyme to **0.05 ng/µl** with 1x Caspase-7 Assay Buffer (20 µl/well).

12. Initiate the reaction by adding 20 µl of diluted capsase-7 enzyme to the wells designated “Positive Control”, “Control Inhibitor” and "Test Inhibitor".

13. Add 20 µl of 1x Caspase-7 Assay Buffer to the “Blank” wells.

14. Incubate at Room Temperature for 30 minutes.

15. Immediately read in a fluorimeter or a microplate reader capable of excitation at λ=400 nm and detection at λ=505 nm. Fluorescence measurements may be read over time to determine enzyme kinetics.

16. The “Blank” value is subtracted from all other readings.

Component	**Blank**	**Positive Control**	**Control Inhibitor**	**Test Inhibitor**
Master Mix	25 µl	25 µl	25 µl	25 µl
Test Inhibitor	-	-	-	5 µl
Diluted Ac-DNLD-CHO	-	-	5 µl	-
Diluent Solution	5 µl	5 µl	-	-
1x Caspase-7 Assay Buffer	20 µl	-	-	-
Diluted Caspase-7 (0.05 ng/µl)	-	20 µl	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

Example Results

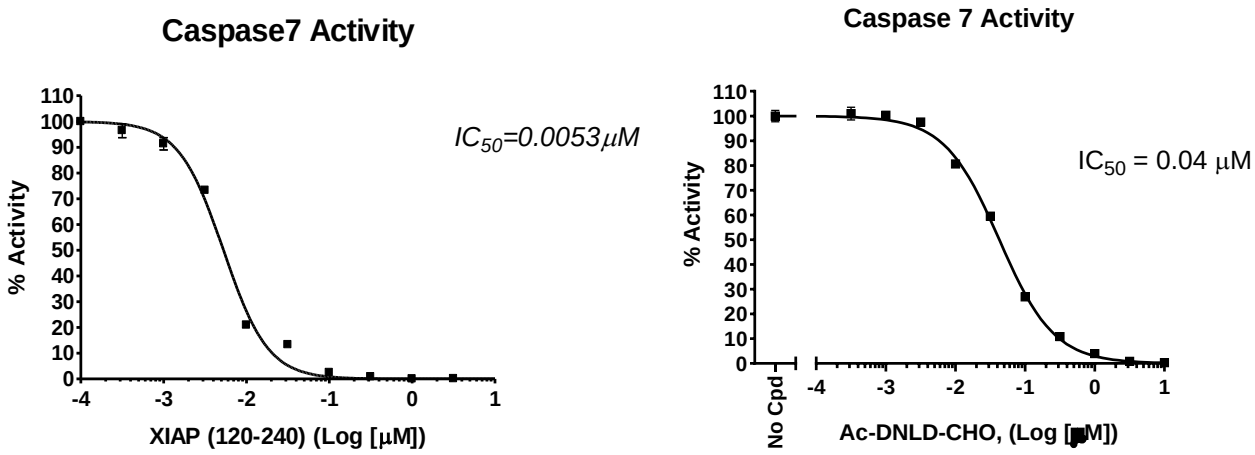

Figure 2: Inhibition of caspase-7 activity by XIAP Bir2 domain and Ac-DNLD-CHO.
Caspase-7 activity was measured in the presence of increasing concentrations of XIAP Bir2 Domain (left panel) and Ac-DNLD-CHO (right panel). The "Blank" value was subtracted from all other values. Results are expressed as the percent of control (activity in the absence of inhibitor, set at 100%).

Data shown is representative.

Troubleshooting Guide

Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For lot-specific information and all other questions, please visit https://bpsbioscience.com/contact.

References

Chai J., *et al.,* 2001, *Cell* 104(5):769-80.
Denault J.B. and Salvesen GS., 2003 *J. Biol. Chem*., 278(36):34042-50.
Boice A. and Bouchier-Hayes L., 2002, *Biochimica et Biophysica Acta* 1867 (6): 118688.
Lee D., *et al.*, 2000 *Molecular Basis of Cell and Developmental Biology* 275(21):16007-16014.

Related Products

Products	*Catalog #*	*Size*
Caspase-8 Homogeneous Assay Kit	80704	96 reactions
Caspase-3 Homogeneous Assay Kit	80700	96 reactions
Caspase-6 Homogeneous Assay Kit	80703	96 reactions
Caspase-3/7 Inhibitor I	27741	5 mg/10 mg/25 mg
Z-DEV-FMK	27314	5 mg
Ac-DEVD-CMK	27315	5 mg

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