GSI Caspase-9 Colorimetric Assay Kit DataSheet

Caspase-9 is an initiator caspase, encoded by the *CASP9* gene. *CASP9* orthologs have been identified in all mammals for which complete genome data are available. The aspartic acid specific protease caspase-9 has been linked to the mitochondrial death pathway. It is activated during programmed cell death (apoptosis). Induction of stress signaling pathways JNK/SAPK causes release of cytochrome c from mitochondria and activation of apaf-1 (apoptosome), which in turn cleaves the pro-enzyme of caspase-9 into the active form. Once initiated caspase-9 goes on to cleave procaspase-3 & procaspase-7, which cleave several cellular targets, including poly ADP ribose polymerase. Caspase-9 precursor procaspase-9 is present as an inactive monomer before it undergoes a conformational change to a dimer and associates with the apf-1 and cytochrome c complex to form an apoptosome.[1] Caspase-9 is involved in the activation cascade of caspases responsible for apoptosis execution. Binding of caspase-9 to Apaf-1 leads to activation of the protease which then cleaves and activates caspase-3. It promotes DNA damage-induced apoptosis in a ABL1/c-Abl-dependent manner and proteolytically cleaves poly(ADP-ribose) polymerase (PARP). This protein can undergo autoproteolytic processing and activation by the apoptosome, a protein complex of cytochrome c and the apoptotic peptidase activating factor 1; this step is thought to be one of the earliest in the caspase activation cascade. Caspase-9 is thought to play a central role in apoptosis and to be a tumor suppressor. Caspase-9 is activated in a cytochrome *c*-independent manner early during TNFα-induced apoptosis in murine cells.[2] Caspase-9 interacts with APAF,[3] BIRC2,[4] caspase-8,[5] NLRP1,[6] and XIAP.[7]

References

1. Bratton, Shawn; Salvesen, Guy (2010). Journal of Cell Science. 123: 3209–3214.
2. McDonnell MA et a. (2003) Cell Death and Differentiation 10:1005–1015.
3. Chu ZL, et al. (2001). J. Biol. Chem. 276 (12): 9239–45.
4. Deveraux QL, et al. (1998). EMBO J. 17 (8): 2215–23.
5. Guo Y, et al. (2002). J. Biol. Chem. 277 (16): 13430–7.
6. Hlaing T, et al. (2001). J. Biol. Chem. 276 (12): 9230–8.
7. Rual JF, et al. (2005). Nature. 437 (7062): 1173–8.

PRINCIPLE OF THE ASSAY

Caspase-9 has a substrate specificity by recognizing tetra-peptide motif x-x-x-Asp. The C-terminal Asp is absolutely required while variations at other three positions can be tolerated. Caspase-9 cleave a variety of cellular substrates after aspartic acid residues-a characteristic that is central to their role in mammalian apoptosis. The Caspase-9 Colormetric Assay Kit provides a simple and convenient means for assaying the activity of caspases that recognize the tetrapeptide sequence LEHD. The assay is based on spectrophotometric detection of the chromophore p-nitroaniline (p-NA) after cleavage from the p-NA-labeled Ac-LEHD-pNA. The p-NA light emission can be quantified using a spectrophotometer or a microtiter plate reader at 400-410nm. Comparison of the absorbance of p-NA from an apoptotic sample with an uninduced control allows determination of the fold increase in caspase-9 activities.

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This package insert must be read in its entirety before using this product.

Storage

Store at 4°C as supplied for 6 months. Store at -20°C after reconstitution for up to 3 months.

MATERIALS PROVIDED

Description	Quantity	Description	Quantity	Description	Quantity
Substrate	1	2 x Assay Buffer	1	200 mM DTT	1
DataSheet/Manual	1				

Bring all reagents to room temperature before use.

Reagent Preparations

2 x Assay Buffer, 20 mL- Dilute to 1 x Assay Buffer with deionized and distill water prior to use.
Substrate: Reconstitute in 1.1 mL of DMSO and dissolve completely prior to use and store at -20°C.
200 mM DTT: Reconstitute in 0.55 mL of deionized and distill water prior to use and store at -20°C.

For Research Use Only

Related products

GR107005 GSI Caspase-3/7 Colorimetric Assay Kit
GR107006 GSI Caspase-3/7 Fluorometric Assay Kit
GR107007 GSI Caspase-1 Colorimetric Assay Kit
GR107008 GSI Caspase-1 Fluorometric Assay Kit
GR107028 GSI Caspase-9 Fluorometric Assay Kit
GR107017 GSI Caspase-2 Colorimetric Assay Kit
GR107018 GSI Caspase-2 Fluorometric Assay Kit
GR107019 GSI Caspase-4 Colorimetric Assay Kit
GR107020 GSI Caspase-4 Fluorometric Assay Kit
GR107021 GSI Caspase-5 Colorimetric Assay Kit
GR107022 GSI Caspase-5 Fluorometric Assay Kit
GR107023 GSI Caspase-6 Colorimetric Assay Kit
GR107024 GSI Caspase-6 Fluorometric Assay Kit
GR107025 GSI Caspase-8 Colorimetric Assay Kit
GR107026 GSI Caspase-8 Fluorometric Assay Kit
GR107029 GSI Caspase-10 Colorimetric Assay Kit
GR107030 GSI Caspase-10 Fluorometric Assay Kit

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Assay Procedure

1. Treat cells by desired method. We recommend conducting four reactions (1) non-induced cells, (2) non-induced cells + inhibitor, (3) induced cells, and (4) induced cells + inhibitor as well as two control reactions (1) caspase-9 positive control, and (2) caspase-9 positive control + inhibitor.
2. Count cells and pellet 1 x 10^6 cells per test in 1.5 ml tube.
3. Resuspend cells in 100 µl of chilled 1 x Assay Buffer and incubate cells on ice for 10 minutes.
4. Centrifuge at 10,000 x g for 1 min.
5. Transfer supernatant (cytosolic extract) to each well of 96-well plate (or a fresh tube if a spectrophotometer is later used) and put on ice for immediate assay (or store at -80°C for future use).
6. Immediately before use, prepare sufficient working reagents per assay: 10 µl of Substrate, 5 µl of 200 mM DTT and 85 µl of 1 x Assay Buffer.
7. Add 100 µl of the Working Reagents prepared as above to each well of 96-well plate.
8. Seal the plate with plate sealer. Incubate at 37°C for 1~2 hours and protect from light.
9. Read the pate at 405 nm (wavelength can range from 400 to 410 nm) in a microplate reader (or a spectrophotometer if microtubes are used).
10. Fold-increase in caspase-9 activities can be determined by comparing the OD_{405} with that of the uninduced control or other desired controls.

Precaution and Technical Notes

1. It is critical to follow the procedure step by step otherwise appropriate color development may not occur as expected.
2. A negative control without addition of substrate is recommended and the OD405 subtraction should be applied to each test to correct the background signal.
3. This kit should not be used beyond the expiration date on the label.
4. Use a fresh reagent reservoir and pipette tips for each step.
5. It is recommended that all controls and samples be assayed in duplicate.
6. Avoid microbial contamination of reagents and buffers. This may interfere with the sensitivity of the assay.