GSI Caspase-8 Colorimetric Assay Kit DataSheet

Caspase-8 is a caspase protein, encoded by the CASP8 gene. It most likely acts upon caspase-3. This protein is involved in the programmed cell death induced by Fas and various apoptotic stimuli. The N-terminal FADD-like death effector domain of this protein suggests that it may interact with Fas-interacting protein FADD. This protein was detected in the insoluble fraction of the affected brain region from Huntington disease patients but not in those from normal controls, which implicated the role in neurodegenerative diseases. Many alternatively spliced transcript variants encoding different isoforms have been described, although not all variants have had their full-length sequences determined.[4] Mutation in caspase 8 gene causes caspase eight deficiency state (CEDS) that has features similar to ALPS, another genetic disease of apoptosis, with the addition of an immunodeficient phenotype. Thus, the clinical manifestations include splenomegaly and lymphadenopathy, in addition to recurrent sinopulmonary infections, recurrent mucocutaneous herpesvirus, persistent warts and molluscum contagiosum infections, and hypogammaglobulinemia. There is sometimes lymphocytic infiltrative disease in parenchymal organs, but autoimmunity is minimal and lymphoma has not been observed in the CEDS patients. CEDS is inherited in an autosomal recessive manner.[1] The clinical phenotype of CEDS patients represented a paradox since caspase-8 was considered to be chiefly a proapoptotic protease, that was mainly involved in signal transduction from TNF family death receptors such as Fas. The defect in lymphocyte activation and protective immunity suggested that caspase-8 had additional signaling roles in lymphocytes. Further work revealed that caspase-8 was essential for the induction of the transcription factor "nuclear factor κB" (NF-κB) after stimulation through antigen receptors, Fc receptors, or Toll-like receptor 4 in T, B, and natural killer cells.[1] Biochemically, caspase-8 was found to enter the complex of the inhibitor of NF-κB kinase (IKK) with the upstream Bcl10-MALT1 adapter complex which were crucial for the induction of nuclear translocation of NF-κB. Moreover, the biochemical form of caspase-8 differed in the two pathways. For the death pathway, the caspase-8 zymogen is cleaved into subunits that assemble to form the mature, highly active caspase heterotetramer whereas for the activation pathway, the zymogen appears to remain intact perhaps to limit its proteolytic function but enhance its capability as an adapter protein.[1] Caspase 8 interacts with numerous molecules such as BCAP31, Bcl-2,[2] CFLAR,[3] DEDD,[4] FADD,[5] and other caspases.[6]

References

1. Chun HJ, et al. (2002). Nature. 419 (6905): 395–9.
2. Ng FW, et al. (1997). J. Cell Biol. 139 (2): 327–38.
3. Micheau O, et al. (2002). J. Biol. Chem. 277 (47): 45162–71.
4. Stegh AH, et al. (1998). EMBO J. 17 (20): 5974–86.
5. Boldin MP, et al. (1996). Cell. 85 (6): 803–15.
6. Guo Y, et al. (2002). J. Biol. Chem. 277 (16): 13430–7.

PRINCIPLE OF THE ASSAY

Caspase-8 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-8 cleave a variety of cellular substrates after aspartic acid residues-a characteristic that is central to their role in mammalian apoptosis. The Caspase-8 Colormetric Assay Kit provides a simple and convenient means for assaying the activity of caspases that recognize the tetrapeptide sequence IETD. The assay is based on spectrophotometric detection of the chromophore p-nitroaniline (p-NA) after cleavage from the p-NA-labeled Ac-IETD-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-8 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
GR107026 GSI Caspase-8 Fluorometric Assay Kit
GR107017 GSI Caspase-2 Colorimetric Assay Kit
GR107018 GSI Caspase-2 Fluorometric Assay Kit
GR107019 GSI Caspase-4 Colorimetric Assay Kit
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GR107022 GSI Caspase-5 Fluorometric Assay Kit
GR107023 GSI Caspase-6 Colorimetric Assay Kit
GR107024 GSI Caspase-6 Fluorometric Assay Kit
GR107027 GSI Caspase-9 Colorimetric Assay Kit
GR107028 GSI Caspase-9 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-8 positive control, and (2) caspase-8 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-8 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.