

GSI Caspase-3/7 Colormetric Assay Kit DataSheet

Caspase-3 is a caspase protein that interacts with caspase-8 and caspase-9. It is encoded by the CASP3 gene and is formed from a 32 kDa zymogen that is cleaved into 17 kDa and 12 kDa subunits. The CASP3 protein is a member of the cysteine-aspartic acid protease (caspase) family.^[1] Sequential activation of caspases plays a central role in the execution-phase of cell apoptosis. Caspases exist as inactive proenzymes that undergo proteolytic processing at conserved aspartic residues to produce two subunits, large and small, that dimerize to form the active enzyme. This protein cleaves and activates caspases 6 and 7; and the protein itself is processed and activated by caspases 8, 9, and 10. It is the predominant caspase involved in the cleavage of amyloid-beta 4A precursor protein, which is associated with neuronal death in Alzheimer's disease.^[2] Caspase-3 is activated in the apoptotic cell both by extrinsic (death ligand) and intrinsic (mitochondrial) pathways.^[3] Caspase-3 functions to inhibit XIAP activity by cleaving caspase-9 at a specific site, preventing XIAP from being able to bind to inhibit caspase-9 activity.^[4] Caspase-3 has a typical role in apoptosis, where it is responsible for chromatin condensation and DNA fragmentation.^[5] Elevated levels of a fragment of Caspase-3, p17, in the bloodstream is a sign of a recent myocardial infarction.^[6] Caspase-3 may play a role in embryonic and hematopoietic stem cell differentiation. Caspase-7, apoptosis-related cysteine peptidase, also known as CASP7, is a human protein encoded by the CASP7 gene. Caspase-7 is a member of the caspase (cysteine aspartate protease) family, and has been shown to be an executioner protein of apoptosis. The precursor of this caspase is cleaved by caspase 3, caspase 10, and caspase 9. It is activated upon cell death stimuli and induces apoptosis. Caspase 7 has been shown to interact with Caspase 8,^[7] Survivin^[8] and XIAP.^[9]

References

1. Alnemri ES, et al. (1996). "Human ICE/CED-3 protease nomenclature". *Cell*. 87 (2): 171.
2. Gervais FG, et al. (1999). *Cell*. 97 (3): 395–406.
3. Salvesen GS (2002). *Cell Death and Differentiation*. 9 (1): 3–5.
4. Denault JB, et al. (2007). *The Biochemical Journal*. 405 (1): 11–9.
5. Porter AG, Jänicke RU (1999). *Cell Death and Differentiation*. 6 (2): 99–104.
6. Agosto M, et al. (2011). *Journal of the American College of Cardiology*. 57 (2): 220–1.
7. Srinivasula SM, et al. (1996). *Proc. Natl. Acad. Sci. U.S.A.* 93 (25): 14486–91.
8. Shin S, et al. (2001). *Biochemistry*. 40 (4): 1117–23.
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PRINCIPLE OF THE ASSAY

Caspase 3 and caspase 7 share similar substrate specificity by recognizing tetra-peptide motif Asp-x-x-Asp.^[12] The C-terminal Asp is absolutely required while variations at other three positions can be tolerated.^[13] Caspase-3 and 7 cleave a variety of cellular substrates after aspartic acid residues—a characteristic that is central to their role in mammalian apoptosis. The Caspase-3/7 Colormetric Assay Kit provides a simple and convenient means for assaying the activity of caspases that recognize the tetrapeptide sequence DEVD. The assay is based on spectrophotometric detection of the chromophore p-nitroaniline (p-NA) after cleavage from the p-NA-labeled Ac-DEVD-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-3/7 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

GR107006 GSI Caspase-3/7 Fluorometric Assay Kit
GR107007 GSI Caspase-1 Colorimetric Assay Kit
GR107008 GSI Caspase-1 Fluorometric Assay Kit
GR107017 GSI Caspase-2 Colorimetric Assay Kit
GR107018 GSI Caspase-2 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 3/7 positive control, and (2) caspase 3/7 positive control + inhibitor.
2. Count cells and pellet 1×10^6 cells per test in 1.5 ml tube.
3. Resuspend cells in 100 μ l 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 μ l of Substrate, 5 μ l of 200 mM DTT and 85 μ l of 1 x Assay Buffer.
7. Add 100 μ l 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 plate 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-3/7 activities can be determined by comparing the OD₄₀₅ 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.

DECLARATION

THIS REAGENT IS FOR IN VITRO LABORATORY TESTING AND RESEARCH USE ONLY. DO NOT USE IT FOR CLINICAL DIAGNOSTICS. DO NOT USE OR INJECT IT IN HUMAN AND ANIMALS. WE ARE NOT RESPONSIBLE FOR ANY CONSEQUENCE OF USING IT IN HUMANS AND ANIMALS.

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