

GSI Caspase-10 Colorimetric Assay Kit DataSheet

Caspase-10 is an enzyme that, in humans, is encoded by the CASP10 gene. Caspase 10 is a member of the cysteine-aspartic acid protease (**caspase**) family. Caspase 10 is involved in the activation cascade of caspases responsible for apoptosis execution. It is recruited to both Fas- and TNFR-1 receptors in a FADD dependent manner. It may participate in the granzyme B apoptotic pathways. Caspase-10 cleaves and activates caspase-3, -4, -6, -7, -8, and -9. It hydrolyzes the small- molecule substrates, Tyr-Val-Ala-Asp-|-AMC and Asp-Glu-Val-Asp-|-AMC.^[1] Isoform 7 can enhance NF-kappa B activity but promotes only slight apoptosis.^[2] Like Caspase-8, caspase-10 is an apical caspases and is involved in the extrinsic death receptor pathway in humans and caspase-10 follows the proximity-induced dimerization model for apical caspases.^[3] Both cleaved and uncleaved caspase-10 may have a pro-death role.^[3] Individual caspase-10 isoforms play distinct and opposing roles in the initiation of death receptor-mediated tumour cell apoptosis.^[4] Caspase-10 can function independently of caspase-8 in initiating Fas- and tumor necrosis factor-related apoptosis-inducing ligand-receptor-mediated apoptosis.^[1] Moreover, Fas crosslinking in primary human T cells leads to the recruitment and activation of caspase-10. Fluorescent resonance energy transfer analysis indicates that the death-effector domains of caspase-8 and -10 both interact with the death-effector domain of FADD.^[1] Nonetheless, caspase-8 and -10 may have different apoptosis substrates and therefore potentially distinct roles in death receptor signaling or other cellular processes.^[1]

References

1. Wang J et al. (2001) Proc Natl Acad Sci USA 98:13884-13888.
2. Wang H et al. (2007) Biochim Biophys Acta 1770:1528-1537.
3. Wachmann K et al. (2010) Biochemistry 49(38): 8307-8315.
4. Mühlethaler-Mottet A et al. (2011) *Cell Death and Disease* 2, e125.

PRINCIPLE OF THE ASSAY

Caspase-10 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-10 cleave a variety of cellular substrates after aspartic acid residues-a characteristic that is central to their role in mammalian apoptosis. The Caspase-10 Colorimetric Assay Kit provides a simple and convenient means for assaying the activity of caspases that recognize the tetrapeptide sequence AEVD. The assay is based on spectrophotometric detection of the chromophore p-nitroaniline (p-NA) after cleavage from the p-NA-labeled Ac-AEVD-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-10 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
GR107030 GSI Caspase-10 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
GR107027 GSI Caspase-9 Colorimetric Assay Kit
GR107028 GSI Caspase-9 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-10 positive control, and (2) caspase-10 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-10 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 OD_{405} 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.