GSI Caspase-5 Colorimetric Assay Kit DataSheet

Caspase 5 is an enzyme that proteolytically cleaves other proteins at an aspartic acid residue, and belongs to a family of cysteine proteases called caspases. It is an inflammatory caspase, along with caspase 1, caspase 4 and the murine caspase 4 homolog caspase 11, and has a role in the immune system.[1] Like other caspases, Caspase 5 plays a role in Pyroptosis, a type of programmed cell death and an inflammatory factor. Pyroptosis is a form of programmed cell death that inherently induces an immune response. It is morphologically distinct from other types of cell death – cells swell up, rupture and release pro-inflammatory cellular contents. This is done in response to a range of stimuli including microbial infections as well as heart attacks (myocardial infarctions).[2] Caspase-1, Caspase-4 and Caspase-5 in humans whereas Caspase-1 and Caspase-11 in mice play important roles in inducing cell death by Pyroptosis. This could lead to limiting the life and proliferation time of intracellular and extracellular pathogens. Caspase-4 and -5 in humans, and Caspase-11 in mice have a unique role as a receptor, whereby it binds to LPS, a molecule abundant in gram negative bacteria. This can lead to the processing and secretion of IL-1β and IL-18 cytokines by activating Caspase-1; this downstream effect is the same as described above. It also leads to the secretion of another inflammatory cytokine that is not processed. This is called pro-IL1α.[3] There is also evidence of an inflammatory caspase, caspase-11 aiding cytokine secretion; this is done by inactivating a membrane channel that blocks IL-1β secretion[3] Caspases can also induce an inflammatory response on a transcriptional level. There is evidence where it promotes transcription of nuclear factor-κB (NF-κB), a transcription factor that assists in transcribing inflammatory cytokines such as IFNs, TNF, IL-6 and IL-8. For example, Caspase-1 activates Caspase-7, which in turn cleaves the poly (ADP) ribose – this activates transcription of NF-κB controlled genes.[4]

References

1. *Martinon F, Tschopp J (2007). Cell Death Differ. 14 (1): 10–22.*
2. Bergsbaken T, et al. (2009) Nature Reviews Microbiology. 7 (2): 99–109.
3. Eldridge MJG; et al. (2015) Current Opinion in Microbiology. 23: 32–41.
4. *Sollberger G, et al. (2014). Innate Immunity. 20 (2): 115–125.*

PRINCIPLE OF THE ASSAY

Caspase-5 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-5 cleave a variety of cellular substrates after aspartic acid residues-a characteristic that is central to their role in mammalian apoptosis. The Caspase-5 Colormetric Assay Kit provides a simple and convenient means for assaying the activity of caspases that recognize the tetrapeptide sequence WEHD. The assay is based on spectrophotometric detection of the chromophore p-nitroaniline (p-NA) after cleavage from the p-NA-labeled Ac-WEHD-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-5 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
GR107022 GSI Caspase-5 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
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
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GR107029 GSI Caspase-10 Colorimetric 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-5 positive control, and (2) caspase-5 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-5 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.