CREATIVE BIOMART®
Assay Kit

Caspase-3 assay Kit for drug discovery

Product Information

Cat.No. Kit-0161

Product Overview

The CASPASE-3 Assay Kit for Drug Discovery is a complete assay system designed to screen caspase-3 inhibitors. It contains both a colorimetric substrate (DEVD-pNA) and a fluorogenic substrate (DEVD-AMC). Cleavage of the p-nitroanilide (pNA) from the colorimetric substrate increases absorption at 405nm. The fluorescent assay is based on the cleavage of 7-amino-4-methylcoumarin (AMC) dye from the C-terminus of the peptide substrate. Cleavage of the dye from the substrate increases its fluorescence intensity at 460 nm. The assays are performed in a convenient 96-well microplate format. The kit is useful to screen inhibitors of caspase-3, a potential therapeutic target. An inhibitor, DEVD-CHO (aldehyde), is also included as a prototypic control inhibitor. The DEVD amino acid sequence is derived from the caspase-3 cleavage site in PARP.

Size

96 wells

Description

Caspase-3 (also known as CPP32, apopain and Yama) is a member of the interleukin-1β converting enzyme (ICE) family of cysteine proteases. The enzyme is composed of 17 and 12 kDa subunits derived from a common proenzyme, pro-caspase-3. Caspase-3 is activated during apoptotic signaling events by upstream proteases including caspase-6, caspase-8 (FLICE) and cytotoxic T-cell-derived granzyme B. Targets of caspase-3 cleavage include poly(ADP-ribose) polymerase (PARP), nuclear lamins and others.

Storage

Store all components except the microplate at -70°C for the highest stability. The caspase-3 enzyme component must be handled particularly carefully in order to retain maximal enzymatic activity. Defrost it quickly in a RT water bath or by rubbing between fingers, then immediately store on an ice bath. The remaining unused enzyme should be quickly refrozen by placing at -70°C. The enzyme is stable to freeze/thaw cycles x 4. To minimize the number of freeze/thaw cycles, aliquot the

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caspase-3 into separate tubes and store at -70°C.

Kit Components

1. Caspase-3 enzyme (human) (recombinant)FORM: 100 U/µl in assay buffer. One U=1 pmol/min at 30°C, 200 µM DEVD-pNA. Purity >95% by SDSPAGESTORAGE: -70°C; AVOID FREEZE/THAW CYCLES!QUANTITY: 5000 U (50 µl) 2. pNA SUBSTRATE (Ac-DEVD-pNA; MW=637)FORM: 2 mM (1.3 mg/ml) in assay bufferSTORAGE: -70°CQUANTITY: 1 ml 3. pNA CALIBRATION STANDARD (p-nitroaniline;MW=138.1)FORM: 50 µM in assay buffer. A405nm=0.525 cm-1STORAGE: -70°CQUANTITY: 1 ml 4. AMC SUBSTRATE (Ac-DEVD-AMC; MW=676)FORM: 0.3 mM (0.20 mg/ml) in assay bufferSTORAGE: -70°CQUANTITY: 1 ml 5. AMC CALIBRATION STANDARD (7-amino-4-methylcoumarin; MW=175)FORM: 30 µM in assay bufferSTORAGE: -70°CQUANTITY: 1 ml 6. INHIBITOR (Ac-DEVD-CHO; MW=502)FORM: 0.1 mM (0.05 mg/ml) in DMSO (dimethylsulfoxide)STORAGE: -70°CQUANTITY: 50 µl 7. ASSAY BUFFER (50 mM HEPES, pH 7.4, 100 mM NaCl, 0.1% CHAPS, 10 mM DTT, 1 mM EDTA, 10% glycerol)STORAGE: -70°CQUANTITY: 20 ml 8. 1/2 -VOLUME MICROPLATE1 clear, 96-wellSTORAGE: Room temperature

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