



## Caspase-3 assay Kit for drug discovery

### Product Information

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#### Cat.No.

Kit-0161

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#### Product Overview

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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.

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#### Size

96 wells

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#### Description

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Caspase-3 (also known as CPP32, apopain and Yama) is a member of the interleukin-1&beta; 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.

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#### Storage

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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



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bath. The remaining unused enzyme should be quickly refrozen by placing at  $-70^{\circ}\text{C}$ . The enzyme is stable to freeze/thaw cycles x 4. To minimize the number of freeze/thaw cycles, aliquot the caspase-3 into separate tubes and store at  $-70^{\circ}\text{C}$ .

### Kit Components

1. Caspase-3 enzyme (human) (recombinant)FORM: 100 U/ $\mu\text{l}$  in assay buffer. One U=1 pmol/min at  $30^{\circ}\text{C}$ , 200  $\mu\text{M}$  DEVD-pNA. Purity >95% by SDS-PAGE STORAGE:  $-70^{\circ}\text{C}$ ; AVOID FREEZE/THAW CYCLES! QUANTITY: 5000 U (50  $\mu\text{l}$ ) 2. pNA SUBSTRATE (Ac-DEVD-pNA; MW=637)FORM: 2 mM (1.3 mg/ml) in assay buffer STORAGE:  $-70^{\circ}\text{C}$  QUANTITY: 1 ml 3. pNA CALIBRATION STANDARD (p-nitroaniline; MW=138.1)FORM: 50  $\mu\text{M}$  in assay buffer.  $A_{405\text{nm}}=0.525 \text{ cm}^{-1}$  STORAGE:  $-70^{\circ}\text{C}$  QUANTITY: 1 ml 4. AMC SUBSTRATE (Ac-DEVD-AMC; MW=676)FORM: 0.3 mM (0.20 mg/ml) in assay buffer STORAGE:  $-70^{\circ}\text{C}$  QUANTITY: 1 ml 5. AMC CALIBRATION STANDARD (7-amino-4-methylcoumarin; MW=175)FORM: 30  $\mu\text{M}$  in assay buffer STORAGE:  $-70^{\circ}\text{C}$  QUANTITY: 1 ml 6. INHIBITOR (Ac-DEVD-CHO; MW=502)FORM: 0.1 mM (0.05 mg/ml) in DMSO (dimethylsulfoxide) STORAGE:  $-70^{\circ}\text{C}$  QUANTITY: 50  $\mu\text{l}$  7. ASSAY BUFFER (50 mM HEPES, pH 7.4, 100 mM NaCl, 0.1% CHAPS, 10 mM DTT, 1 mM EDTA, 10% glycerol) STORAGE:  $-70^{\circ}\text{C}$  QUANTITY: 20 ml 8. 1/2 -VOLUME MICROPLATE 1 clear, 96-well STORAGE: Room temperature