Caspase-8 assay Kit for drug discovery

Cat. No. Kit-0172 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	The CASPASE-8 Drug Discovery Kit is a complete assay system designed to screen caspase-8 inhibitors. It contains both a colorimetric substrate (IETD-pNA) and a fluorogenic substrate (IETD-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 microtiter-plate format. The kit is useful to screen inhibitors of caspase-8, a potential therapeutic target. An inhibitor, IETD-CHO (aldehyde), is included for use as a control.
Size	96 wells
Description	Caspase-8 (also known as FLICE, MACH and Mch5) is a member of the interleukin-1β converting enzyme (ICE) family of cysteine proteases. It is implicated as the apical signaling protease in Fas-induced apoptosis. The enzyme is composed of 18 and 11 kDa subunits derived from a common proenzyme. The N-terminal prodomain of the zymogen comprises two "death effector domains" homologous to those of FADD (Mort1). Activation of the Fas-receptor (Apo-1/CD95), by ligand binding, causes FADD to bind the receptor. Procaspase-8 binds the receptor-bound FADD, presumably via interactions between their homologous domains. Binding of the proenzyme leads to its proteolytic processing and activation, possibly by autocatalysis or by interaction with a related caspase (e.g. FLICE2/Mch4). An event which lies downstream from caspase-8 activation is proteolytic activation of the caspase-3 proenzyme, possibly through direct cleavage by caspase-8. The substrate IETD-pNA

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	is based on the recognition sequence for proteolytic activation in procaspase-3. Those tetrapeptide substrates, which incorporate the IETD sequence, are among those cleaved most efficiently by caspase-8.
Storage	Store all components except the microplate at -70°C for the highest stability. The caspase-8 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 caspase-8 into separate tubes and store at -70°C.
Kit Components	1. Caspase-8 enzyme (human) (recombinant)FORM: 100 U/µin assay buffer. One U=1 pmol/min at 30°C, 200 µM IETD-pNA. Purity >95% by SDS-PAGESTORAGE: -70°C; AVOID FREEZE/THAW CYCLES!QUANTITY: 5000 U (50 µl) 2. pNA SUBSTRATE (Ac-IETD-pNA; MW=639)FORM: 2 mM (1.3 mg/ml) in assay bufferSTORAGE: -70°CQUANTITY: 1 ml 3. CALIBRATION STANDARD (p-nitroaniline;MW=138)FORM: 50 µM in assay buffer. A405nm=0.525 cm-1STORAGE: -70°CQUANTITY: 1 ml 4. AMC SUBSTRATE (Ac-IETD-AMC; MW=674)FORM: 0.75 mM (0.5 mg/ml) in assay buffer STORAGE: -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-IETD-CHO; MW=502.5)FORM: 0.1 mM (0.05 mg/ml) in DMSO (dimethylsulfoxide)STORAGE: -70°CQUANTITY: 50 µ7. 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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