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Certificate of Analysis

Caspase 3/7 Assay Kit (Ac-DEVD-AMC Substrate)

Catalog # 17-367
Lot # DAM1400023

Kit Components

2X Caspase Buffer
Catalog # 20-274
Lot # DAM1437181
One vial containing **10.5ml** of 40mM PIPES, pH 7.2; 150mM NaCl, 5mM EDTA, 0.2% CHAPS and 15% sucrose.

Dithiothreitol
Catalog # 20-273
Lot # DAM1437180
Two vials, each containing **15.4mg** of lyophilized dithiothreitol.

White flat bottom 96-well plate
Two 96 well white plates.

Caspase Substrate
Catalog # 12-541
Lot # DAM1441053
One vial containing **200µl** of a proprietary cocktail of Ac-DEVD-AMC substrate in DMSO. **Protect Caspase Substrate solution from light.**

Caspase 3, active
Catalog # 14-264
Lot # 26083
One vial containing **20µg** of recombinant full length protein in **200µl** phosphate buffered saline (PBS) containing 50% glycerol. The recombinant full length protein contains a C-terminal histidine tag; expressed in *E. coli*.

FOR RESEARCH USE ONLY
NOT RECOMMENDED OR INTENDED FOR DIAGNOSIS OF DISEASE IN HUMANS
DO NOT USE IN HUMANS

Kit Description

Quantity: Sufficient reagents for 192 assays per kit.

Storage: The 96 well plates should be stored at room temperature. All other components are stored at -20°C.

Stability: Components are stable for 6 months from date of shipment if stored and handled correctly.

Use: This assay is a simple homogeneous assay performed in a microtiter plate. Caspase 3/7 activity is measured from the release of a fluorophore (AMC) from the substrate Ac-DEVD-AMC.

Please refer to the User Manual for further information and a detailed assay procedure.

Quality Control Testing

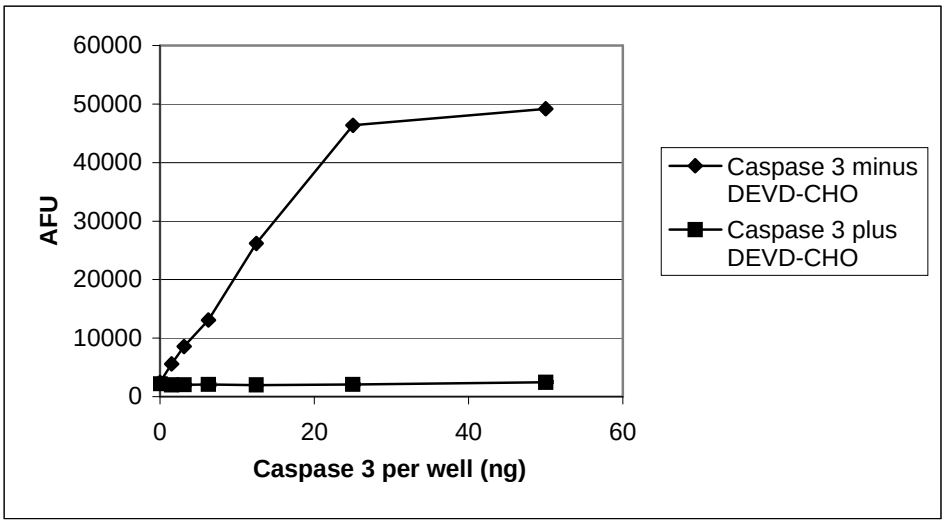

Figure 1. Ac-DEVD-CHO inhibition of Caspase 3 Activity. Two fold serial dilutions of the Caspase 3, active were incubated with the caspase 3 substrate (Ac-DEVD-AMC) at 30°C in the presence and absence of 25µM caspase 3 inhibitor (Ac-DEVD-CHO). Fluorescence was measured after 30 minutes using a Wallac Victor^2 1420, (PerkinElmer), Excitation = 380nm, Emission = 460nm. Arbitrary Fluorescent Units (AFU) are plotted. Points are means of triplicates.

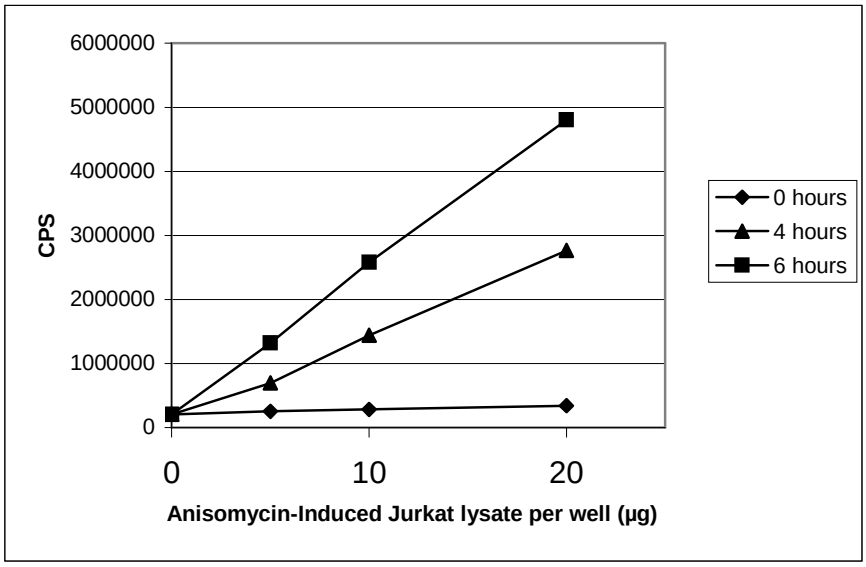

Figure 2. Detection of Caspase 3/7 activity in anisomycin-induced Jurkat cells using Ac-DEVD-AMC substrate. Indicated concentrations of lysate prepared from Jurkat cells treated with 200µM anisomycin for 0, 4, or 6 hours were incubated at 37°C with Caspase Assay Substrate (Ac-DEVD-AMC). Fluorescence was measured after 30 minutes using an Analyst AD Multimode Reader (Molecular Devices), Excitation = 360nm, Emission = 460nm. Counts per second (CPS) are plotted. Points are means of duplicates.

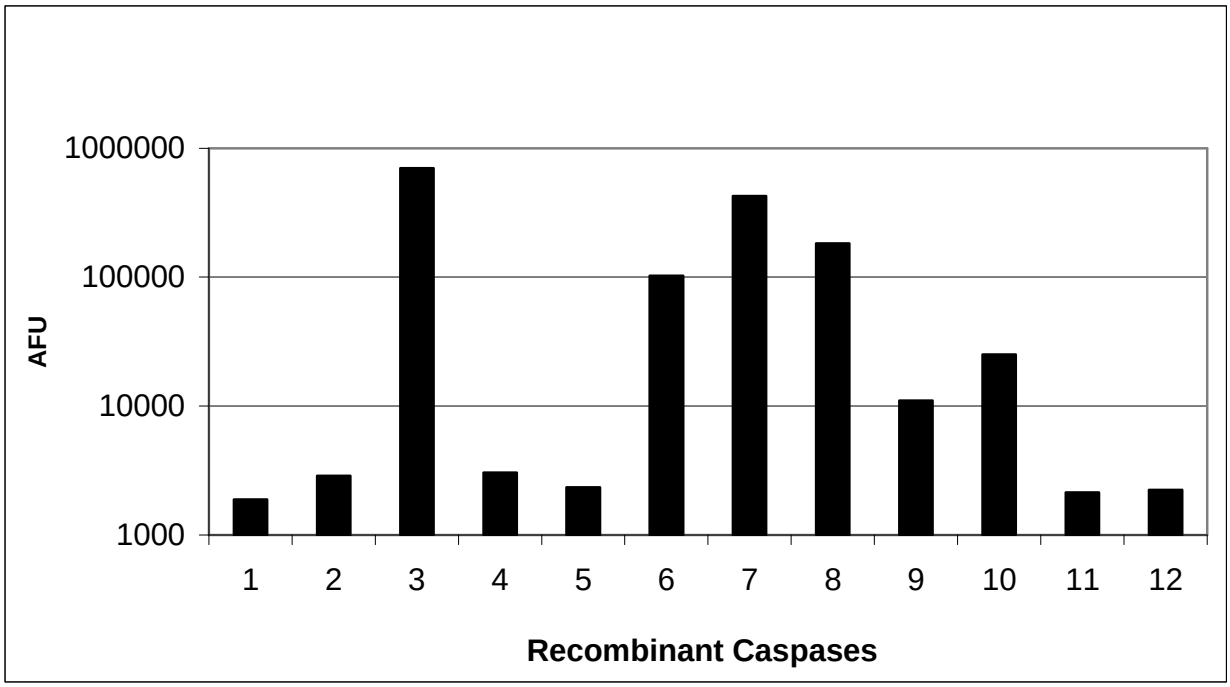

Figure 3. Activities of Caspases 1-12 Measured Using Ac-DEVD-AMC Substrate. Two units each of recombinant caspases 1-12 were incubated (30°C) for 30 minutes. Fluorescence was measured using a Wallac Victor^2 1420, (PerkinElmer), Excitation = 380nm, Emission = 460nm. Arbitrary Fluorescent Units (AFU) are plotted. Points are means of triplicates.