Caspase-3 Assay Kit (Fluorometric)

Cat. No. KITG-150 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	For the rapid, sensitive and accurate measurement of Caspase-3 Activity in cell and tissue lysates.
Description	Description The assay is based on detection of cleavage of substrate DEVD-AFC (AFC: 7-amino-4-trifluoromethyl coumarin). DEVD-AFC emits blue light (λ_{max} = 400 nm); upon cleavage of the substrate by Caspase-3 or related caspases, free AFC emits a yellow-green fluorescence (λ_{max} = 505 nm), which can be quantified using a fluorometer or a fluorescence microtiter plate reader. Comparison of the fluorescence of AFC from an apoptotic sample with an un-induced control allows determination of the fold increase in Caspase-3 activity. Scientific Background Three major factors need to be taken into account when using caspase activity assays: 1. The substrate in a particular assay is not necessarily specific to a particular caspase. Cleavage specificities overlap so reliance on a single substrate/assay is not recommended. Other assays, such as Western blot or use of fluorescent substrates, e.g., FRET assays, should be used in combination with caspase activity assays. 2. The expression and abundance of each caspase in a particular cell type and cell line will vary. 3. As the activation and cleavage of caspases in the cascade will change over time, you should consider when particular caspase will be at its peak concentration, e.g., after 3 hours, after 20 hours etc. Known cross-reactivities with other caspases: Apoptotic Executer Caspases Caspase-3 — Cleavage motif: DEVD; Inhibitor motif: DEVD, LEHD*, IETD, LETD; Cross-reactivity with other caspases: Caspase-2 and Caspase-6. Caspase-6 — Cleavage motif: VEID; Inhibitor motif: DEVD, LEHD*, IETD, LETD; Cross-reactivity with other caspases: Caspase-3. Caspa

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	se-7 — Cleavage motif: DEVD; Inhibitor motif: DEVD, LEHD*, IETD, LETD; Cross-reactivity with other caspases: Caspase-1 and Caspase-3. * inhibits at high concentration. Classification of caspases based on synthetic substrate preference, does not reflect the real caspase substrate preference in vivo and may provide inaccurate information for discriminating amongst caspase activities. Thus, caution is advised in applying the intrinsic tetrapeptide preferences to predict the targets of individual caspases. Kit Components Lysis Buffer IV — 100 mL 2X Reaction Buffer I — 4 × 2 mL DEVD-AFC (1 mM) — 500 µL DTT I — 400 µL Materials Required but Not Supplied Microcentrifuge Pipettes and pipette tips Fluorometric microplate reader or Fluorometer Black 96-well plate Orbital shaker Compatible Sample Types Cell lysates and tissue lysates. Features & Benefits For the rapid, sensitive and accurate measurement of Caspase-3 Activity in cell and tissue lysates. Caspase-3 Assay Kit (Fluorometric) provides a simple and convenient means for assaying the DEVD-dependent caspase activity.
Applications	Measurement of Caspase-3 Activity in cell and tissue lysates.
Notes	This product detects proteolytic activity. Do not use protease inhibitors in the sample preparation step as it might interfere with the assay. This product is for research use only and is not intended for diagnostic use. Size: Troubleshooting: Assay not working • Cells did not lyse completely — Re-suspend the cell pellet in the Lysis Buffer IV and incubate as described in the datasheet. • Experiment was not performed at optimal time after apoptosis induction — Perform a time-course induction experiment for apoptosis. • Plate read at incorrect wavelength — Ensure you are using appropriate reader and filter settings (refer to datasheet). • Old DTT used — Always use freshly thawed DTT I in the Lysis Buffer IV. High Background • Increased amount of cell lysate used — Refer to datasheet and use the suggested cell number to prepare lysates. • Increased amounts of components added due to incorrect pipetting — Use calibrated pipettes. • Incubation of cell samples for extended periods — Refer to

datasheet and incubate for exact times. • Use of expired kit or improperly stored reagents — Always check the expiry date and store the individual components appropriately. • Contaminated cells — Check for bacteria/yeast/mycoplasma contamination. Lower signal levels • Cells did not initiate apoptosis — Determine the time-point for initiation of apoptosis after induction (time-course experiment). • Very few cells used for analysis — Refer to datasheet for appropriate cell number. • Use of samples stored for a long time — Use fresh samples or aliquot and store and use within one month for the assay. • Incorrect setting of the equipment used to read samples — Refer to datasheet and use the recommended filter setting. • Allowing the reagents to sit for extended times on ice — Always thaw and prepare fresh reaction mix before use. General Issues • Improperly thawed components — Thaw all components completely and mix gently before use. • Incorrect incubation times or temperatures — Refer to datasheet & verify the correct incubation times and temperatures. • Incorrect volumes used — Use calibrated pipettes and aliquot correctly. • Air bubbles formed in the well/tube — Pipette gently against the wall of the well/tubes. • Substituting reagents from older kits/lots — Use fresh components from the same kit. • Use of a different 96-well plate — Fluorescence: Black plates; Absorbance: Clear plates. Samples with erratic readings • Uneven number of cells seeded in the wells — Seed only equal number of healthy cells (correct passage number). • Samples prepared in a different buffer — Use the Lysis Buffer IV provided in the kit. • Adherent cells dislodged and lost at the time of experiment — Perform experiment gently and in duplicates/triplicates; apoptotic cells may become floaters. • Cell/tissue samples were not completely homogenized — Use Dounce homogenizer (increase the number of strokes); observe efficiency of lysis under microscope. • Samples used after multiple freeze-thaw cycles — Aliquot and freeze samples, if needed to use multiple times. • Presence of interfering substance in the sample — Troubleshoot as needed. • Use of old or inappropriately stored samples — Use fresh samples or store at correct temperatures until use. Unexpected results • Measured at incorrect wavelength — Check the equipment and the filter setting. • Cell samples contain interfering substances — Troubleshoot if it interferes with the kit (run proper

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	controls).
Storage	Store kit at -20°C. Protect DEVD-AFC from light. Store Lysis Buffer IV and 2X Reaction Buffer I at +4°C after opening. All reagents are stable for 6 months under proper storage conditions. Tissue and cell lysates can be kept frozen at -80°C for up to 2 months without significant loss of activity.
Concentration	DEVD-AFC: 1 mM. DTT I stock: 1.0 M.
Reaction conditions	Fluorometric detection of DEVD-AFC cleavage; excitation at 400 nm and emission at 505 nm.
Usage	Preparation For tissue samples, homogenize in Lysis Buffer IV (for 1X volume of tissue, add 3X volume of Lysis Buffer IV) to generate tissue lysate, then follow the kit procedure. Tissue and cell lysates can be kept frozen at -80°C for up to 2 months without significant loss of activity. Aliquot enough 2X Reaction Buffer I for the number of assays to be performed. Add DTT I to the 2X Reaction Buffer I immediately before use (10 mM final concentration: add 10 µL of 1.0 M DTT I stock per 1 mL of 2X Reaction Buffer I). Assay Protocol 1. Induce apoptosis in cells by desired method. Concurrently incubate a control culture without induction. 2. Count cells and pellet 1–5 × 10^6 cells or use 20–200 µg cell lysates (depending on the apoptosis level). 3. Re-suspend cells in 50 µL of chilled Lysis Buffer IV. 4. Incubate cells on ice for 10 minutes. 5. Aliquot enough 2X Reaction Buffer I for the number of assays to be performed. Add DTT I to the 2X Reaction Buffer I immediately before use (10 mM final concentration: add 10 µL of 1.0 M DTT I stock per 1 mL of 2X Reaction Buffer I). Add 50 µL of 2X Reaction Buffer I (containing 10 mM DTT I) to each sample. 6. Add 5 µL of the 1 mM DEVD-AFC substrate (final concentration 50 µM) and incubate at 37°C for 1–2 hour. 7. Read samples in a fluorometer equipped with a 400-nm excitation filter and 505-nm emission filter. You may also perform the entire assay directly in a Black 96-well plate. Fold-increase in Caspase-3 activity can be

	determined by comparing these results with the level of the un-induced control. Assay Time Incubate cells on ice for 10 minutes; incubate with DEVD-AFC substrate at 37°C for 1–2 hour. Analysis Comparison of the fluorescence of AFC from an apoptotic sample with an un-induced control allows determination of the fold increase in Caspase-3 activity.

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