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Caspase 3 Assay kit (Cat #: E-151)

COMPONENTS: 10x Caspase 3 Buffer- 0.6 ml, store at 4°C
50x Caspase 3 Substrate- 100 µl, store at -20°C (for 100 wells; **contains DMSO**)
100x DTT: 0.1 ml, store at -20°C
10x Cell Lysis Solution- 25 ml, store at 4°C (**contains 1% TX-100; swirl bottle briefly prior to dilution**)

PRODUCT DESCRIPTION: . The Caspase 3 activity assay kit is based on proteolytic hydrolysis of the chromogenic peptide substrate Asp-Met-Gln-Asp-p-nitroanilide. Cleavage of nitroanilide from the substrate increases absorbance at 405 nm (extinction coefficient= $9.96 \text{ mM}^{-1}\text{cm}^{-1}$), allowing for sensitive and quantitative assay of Caspase 3 activity present in tissue/cell lysates. Both kinetic and endpoint determinations can be performed. Kit components are stable for at least 1 year if stored and handled properly.

Preparation of cell/tissue extracts:

1. Prepare 1x Cell Lysis Solution by diluting 10x Cell Lysis Solution with ice-cold dH₂O. Bring up at least $\sim 10^5$ washed cells in 100 – 200 µl ice-cold 1x Cell Lysis Solution by pipetting up and down gently. Leave lysate on ice for 5 min with agitation. If lysate is overly turbid, add more 1x Cell Lysis Solution and repeat pipetting. Tissue is homogenized in ice-cold 1x Cell Lysis Solution (~ 10 mg tissue in 0.5 ml).
2. Centrifuge lysate in a cold microfuge at $\sim 14,000$ rpm for 5 min. Supernatant is harvested and stored at -80°C.
3. Use the BCA protein assay method to determine lysate protein concentration. A suggested sample protein concentration range is 1 – 2 mg/ml.

Preparation of working solution:

Each well of a 96-well plate requires 50 µl of freshly prepared working solution. For preparation of 1 ml of working solution, for example, add 100 µl 10x Caspase 3 Buffer to 870 µl ddH₂O followed by addition of 10 µl 100x DTT and 20 µl 50x Caspase 3 Substrate. Mix contents by vortexing and use the working solution immediately.

Enzyme assay (endpoint measurement):

1. Use a plain (uncoated) 96-well plate for the assay. Add 10 µl of each sample to the plate.
2. Calculate the amount of working solution required for each assay, and prepare the solution as described above. Swiftly add 50 µl working solution to each sample well. Mix contents by brief gentle agitation for 10 sec. Blot dry bottom of plate if necessary. IMMEDIATELY measure O.D._{405 nm} using a plate reader. Data are recorded as O.D._{0 min}.
3. Cover plate and incubate at 37°C for 30 or 60 min (do not use CO₂ incubator). Measure O.D._{405 nm} after incubation. Data are recorded as O.D._{30 min} or O.D._{60 min}.
4. If incubation for 30 min, Caspase 3 activity in IU/L = $(\text{O.D.}_{30 \text{ min}} - \text{O.D.}_{0 \text{ min}}) \times 1000 \times 60 \mu\text{l} / (30 \text{ min} \times 0.3 \text{ cm} \times 9.96 \times 10 \mu\text{l}) = (\text{O.D.}_{30 \text{ min}} - \text{O.D.}_{0 \text{ min}}) \times 66.93$. If incubation for 60 min, Caspase 3 activity in IU/L = $(\text{O.D.}_{60 \text{ min}} - \text{O.D.}_{0 \text{ min}}) \times 33.47$

For tissue/cell lysates, enzyme activity should be normalized by protein concentration, and can be presented as units/µg proteins.

Additional information:

- The 50x Caspase 3 Substrate solution contains DMSO. Please refer to the product page of our website or contact us for MSDS information of DMSO.