# Caspase-8 activity detection kit (spectrophotometric method)

BA3150

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| **Storage/Stability:** | Store the kit at -20℃, while store the Lysis Buffer and 2×Reaction Buffer at 4℃ after opened. All reagents will remain stable for one year. |
| **Experimental Methods:** | A. General Considerations<br>• Aliquot enough 2X Reaction Buffer for the number of assays to be performed. Add DTT into the 2X Reaction Buffer immediately to 10 mM final concentration before use (add 10 µl of 1.0 M DTT stock in per 1ml 2X Reaction Buffer).<br>• Store the Lysis Buffer at 4℃ after thawing.<br>• Do not expose Caspase-8 Substrate from light.<br>B. Assay Procedure<br>1.Induce cells apoptosis with required way and set negative control without induction at the same time.<br>2.Collect cells and wash cells twice with PBS by centrifugation in 2000 rpm for 5 min.<br>3.Collect 3～5×106 cells, try to remove PBS<br>4.Add 50µl cold prepared Lysis Buffer into collected cells and mix uniformity. (Note: Before using Lysis Buffer, add 0.5 µl DTT into 50 µl Lysis Buffer)<br>5.Incubate cells on ice for 20～60 min, vortex vibration 3-4 times,10 s per time, or freeze thawing 2-3 times.<br>6.Centrifugation in 10,000 rpm at 4 ℃ for 1 min.<br>7.Suck supernatant and transfer to a new tube, put it on ice<br>8.Get a small quantity of supernatant to assay the protein concentration by Bradford or BCA method.<br>9.Take 50 µl supernatant containing 100～200 µg protein. If there is no enough volume, Lysis Buffer can be compensated. |

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| | 10.Add 50 µl of 2X Reaction Buffer to each sample. (Note: add 0.5 µl DTT into 50 µl 2×Reaction Buffer)<br>11.Add 5 µl Caspase-8 Substrate (200 µM final conc.) and incubate at 37oC for 1～2 hour, away from light.<br>12.Determine extinction value of samples by a spectrophotometer (using a 100 µl micro quartz cuvet) or a microtiter plate reader at 400 nm or 405nm. Get the result of the induced group's Caspase-8 activity by computing ODinducer/ODnegtive control.<br><br>Note: Background of cell lysates buffers from the readings of induced samples and negative control should be subtracted before calculating fold increase in Caspase-8 activity. |