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Caspase-3/7 activity

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We use this protocol and it's working

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Abstract

Detecting cell apoptosis by measuring Caspase-3/7 activation

Protocol for detecting Caspase-3/7 activation

1 Caspase-3/7 substrate FAM-DEVD-FMK (ImmunoChemistry) is reconstituted in DMSO and stored at -20°C. The FAM-DEVD-FMK reagent is one of the fluorochrome-labeled inhibitors of caspases that covalently and irreversibly binds to the active caspases. The green fluorescence intensity is a direct measurement of Caspase-3/7 activity.

2 To test toxic or rescuing effects on cell apoptosis, the cells are pre-treated with DMSO or any pharmacological perturbations.

3 When cells are available, first dilute the 150X stock concentrate 1:5 in PBS to form the staining solution at 30X.

4 Add the 30X staining solution at a dilution of 1:30 to cell culture medium to form 1X staining solution. The solution should be used immediately.

5 Prepare 1X Apoptosis Buffer by diluting 10X Apoptosis Buffer 1:10 in dH2O.

6 Add 100-300 µL FAM-FLICA solution to each well and incubate for 30 min at 37°C.

7 Aspirate the FAM-FLICA staining solution and rinse 1X Apoptosis Buffer once.

8 The cells are fixed by 4% paraformaldehyde (PFA) for 15 min and stained with antibodies and 5 µg/mL Hoechst for confocal microscope imaging.