CaspaTag Caspase Activity Protocol

This assay is based on carboxyfluorescein labeled fluoromethyl ketone (FMK)-peptide inhibitors of caspases.

Experimental Preparation and Setup:

Working Dilution of FMK-peptide inhibitors:

1. Reconstitute lyophilized FMK-peptide in 50 µl of DMSO resulting in a 150X concentration.
2. Mix contents at room temperature until dissolved. Aliquots may be made and stored frozen at –20^{o}C
3. Prior to using, make a 30X Working Dilution. Dilute the 150X substrate 1:5 in PBS, pH 7.4 (1 part 150X FMK-peptide and 4 parts PBS). Mix well.
4. Protect from light at all times.

1X Working Dilution Wash Buffer:

1. Place 10X Wash Buffer in a 37^{o}C water bath for 30 minutes to dissolve precipitated protein and buffer salts.
2. Mix thoroughly.
3. Dilute 10 ml of 10X Wash Buffer in 90 ml of dH_2O and mix thoroughly.

Protocol for Flow Cytometry:

1. Place 300 ul of cells (5 x 10^5 to 1 x 10^6 cells/ml) in a flow tube.
2. Add 10 µl of the 30X Working Dilution FMK-peptide directly to the cell suspension and gently mix
3. Incubate the cells for 1 hour under the appropriate conditions… 37^{o}C, 7% CO_2 protected from light.
4. Add 2 ml of 1X Wash Buffer to the labeled cells.
5. Spin down the cells at 400xg for 5 minutes at room temperature.
6. Remove the supernatant.
7. Resuspend the cells in 2 ml of 1X Wash Buffer and pellet the cells.
8. Resuspend the cells in 400 µl of 1X Wash Buffer.
9. Add 2 µl of PI solution to each sample.
10. Place the cells on ice.
11. Examine by flow cytometry.