Certificate of Analysis

CaspACE™ FITC-VAD-FMK In Situ Marker:

Part No.	Size
G746A	50µl
G746B	125µl

Part# 9PIG746

Revised 9/16

Description: CaspACE™ FITC-VAD-FMK In Situ Marker is a fluoroisothiocyanate (FITC) conjugate of the cell permeable caspase inhibitor VAD-FMK. This structure allows delivery into the cell where it binds to activated caspase, serving as an in situ marker for apoptosis. The bound marker is localized by fluorescence detection.

Formulation: CaspACE™ FITC-VAD-FMK In Situ Marker is supplied as a 5mM solution in DMSO.

Storage Conditions: Store aliquots at –20°C protected from light and moisture, preferably in a desiccator. Avoid multiple freeze-thaw cycles and exposure to frequent temperature changes.

Usage Notes: Concentration gradients may form in frozen products and should be dispersed upon thawing. Mix well prior to use.

Usage Information

Protocol for use of CaspACE™ FITC-VAD-FMK In Situ Marker

1. Seed Jurkat cells at 1×10^5 cells/ml and grow in RPMI-1640 + 10% FBS in a 37°C, 5% CO_2 incubator for 2–3 days, until they reach a density of 5×10^5 cells/ml.
2. Prepare poly-L-lysine-coated slides. A 0.01% poly-L-lysine solution is used to coat each chamber on multi-chamber slides. When partially dry, the slides are rinsed in NANOpure® water and then allowed to air-dry. Poly-L-lysine-coated slides can be prepared in advance and stored at 4°C for up to 7 days prior to use.
3. Add anti-Fas mAb (Clone CH-11, PanVera Cat. #SY-100) to a final concentration of 0.1µg/ml. Do not add to controls. Incubate for 3–4 hours at 37°C.
4. Add CaspACE™ FITC-VAD-FMK In Situ Marker to the Jurkat cells at a final concentration of 10µM. Protect cells from light and incubate for 20 minutes in the incubator. Keep cells protected from light for the remaining steps.
5. Centrifuge at 300 × *g* for 5 minutes.
6. Wash cells with PBS then centrifuge at 300 × *g* for 5 minutes.
7. Suspend cells in PBS to 1.5×10^6 cells/ml.
8. Add cells to poly-L-lysine-coated slides and incubate at room temperature for 5 minutes to allow the cells to adhere to the poly-L-lysine.
9. Fix in 10% buffered formalin for 30 minutes at room temperature (protected from light).
10. Rinse 3 × 5 minutes in PBS.
11. Add mounting medium and cover slips to the slides. Analyze under a fluorescence microscope.

Signed by:

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