ELISA PRODUCT INFORMATION & MANUAL

Active Caspase Staining Kit (Fluorometric)

NBP2-54866

Enzyme-linked Immunosorbent Assay for quantitative detection. For research use only.
Not for diagnostic or therapeutic procedures.

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Table of Contents

1. Overview

Activation of caspases plays a central role in apoptosis. Novus Biologicals's Active Caspase Staining Kit (Fluorometric) provides a convenient means for detecting activated caspases in living cells.

The assay utilizes the caspase inhibitor VAD-FMK conjugated to sulfo-rhodamine (Red-VAD-FMK) as the fluorescent *in situ* marker. Red-VAD-FMK is cell permeable, nontoxic, and irreversibly binds to activated caspases in apoptotic cells. The fluorescence label allows detection of activated caspases in apoptotic cells by fluorescence microscopy, flow cytometry, or fluorescence plate reader.

2. Protocol Summary

Sample Preparation

Analyze by Flow Cytometry

OR

Detect by Fluorescence Microscopy

OR

Measure Fluorescence in Microplate Reader

3. Components and Storage

A. Kit Components

Item	Quantity
Red-VAD-FMK	100 µL
Wash Buffer	2 x 100 mL
Z-VAD-FMK	100 µL

* Store kit at -20°C.

B. Additional Materials Required

- Microcentrifuge
- Pipettes and pipette tips
- Fluorescent microplate reader or microscope
- Flow cytometer
- Black microtiter plate
- Orbital shaker

4. Assay Protocol

1. Staining Procedure

a) Induce apoptosis in cells (1 x 10^6 cells/ml) by the desired method. Concurrently incubate a control culture *without* induction.

An additional control can be prepared by adding the caspase inhibitor Z-VAD-FMK at 1 µl/ml to an induced culture to inhibit caspase activation.

b) Aliquot 300 µl each of the induced and control cultures into eppendorf tubes.

c) Add 1 µl of Red-VAD-FMK into each tube and incubate for 0.5-1 hour at 37°C incubator with 5% CO_2.

d) Centrifuge cells at 3000 rpm for 5 minutes and remove supernatant.

e) Re-suspend cells in 0.5 ml of Wash Buffer and centrifuge again.

f) Repeat step e.

g) Proceed to Step 2, 3 or 4 depending on methods of analysis of the un-induced control.

Note: This product detects proteolytic activity. Do not use protease inhibitors in the sample preparation step as it might interfere with the assay

2. Quantification by Flow Cytometry:

For flow cytometric analysis, re-suspend cells in 300 µl of Wash buffer. Put samples on ice. Analyze samples by flow cytometry using the FL-2 channel (Ex = 540 nm; Em = 570 nm).

3. Detection by Fluorescence Microscopy:

For fluorescence microscopic analysis, re-suspend cells in 100 µl Wash buffer. Put one drop of the cell suspension onto a microslide and cover with a coverslip. Observe cells under a fluorescence microscope using rhodamine filter.

Caspase positive cells appear to have brighter red signals, whereas caspase negative control cells show much weaker signal.

4. Analysis by Fluorescence Plate Reader:

For analysis with fluorescence plate reader, re-suspend cells in 100 µl Wash Buffer and then transfer the cell suspension to each well of the black microtiter plate. Measure the fluorescence intensity at Ex/Em = 540/570 nm. For control, use wells containing unlabeled cells.

Note:

Ex/Em = 488/570 nm will also work, although it’s not an optimal wavelength.

5. Troubleshooting

Problem	Reason	Solution
High Background	Cell density is higher than recommended	Refer to datasheet and use the suggested cell number
	Increased volumes of components added	Use calibrated pipettes accurately
	Incubation of cell samples for extended periods	Refer to datasheets and incubate for exact times
	Use of extremely confluent cells	Perform assay when cells are at 80-95% confluency
	Contaminated cells	Check for bacteria/ yeast/ mycoplasma contamination
Lower signal levels	Cells did not initiate apoptosis	Determine the time-point for initiation of apoptosis after induction (time-course experiment)
	Very few cells used for analysis	Refer to data sheet for appropriate cell number
	Incorrect setting of the equipment used to read samples	Refer to datasheet and use the recommended filter setting
	Use of expired kit or improperly stored reagents	Always check the expiry date and store the components appropriately

Erratic results	Uneven number of cells seeded in the wells	Seed only healthy cells (correct passage number)
	Adherent cells dislodged at the time of experiment	Perform experiment gently and in duplicates or triplicates for each treatment
	Incorrect incubation times or temperatures	Refer to datasheet & verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly