

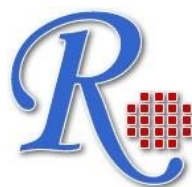
RayBio® CaspGLOW™ Fluorescein Active Caspase-8 Staining Kit

User Manual Version 2.0

June 21, 2021

**RayBio® Caspase-8 Fluorometric Assay
Kit Protocol**

(Cat#: 68FLS-Casp8-S)



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I. INTRODUCTION

Activation of caspases plays a central role in apoptosis. The **CaspGLOW™ Fluorescein Active Caspase-8 Staining Kit** provides a convenient means for sensitive detection of activated caspase-8 in living cells. The assay utilizes the caspase-8 inhibitor, IETD-FMK, conjugated to FITC (FITC-IETD-FMK) as a fluorescent *in situ* marker. FITC-IETD-FMK is cell permeable, nontoxic, and irreversibly binds to activated caspase-8 in apoptotic cells. The FITC label allows detection of activated caspase-8 in apoptotic cells directly by fluorescence microscopy, flow cytometry, or fluorescence plate reader.

II. REAGENTS

Store kit at -20°C

Components	68FLS-Casp8-S25	68FLS-Casp8-S100	Part Number
	25 assays	100 assays	
FITC-IETD-FMK	25 µl	100 µl	Part A
Wash Buffer	50 ml	2 x 100 ml	Part B
Z-VAD-FMK	10 µl	10 µl	Part C

III. CASPASE-8 ASSAY PROTOCOL

A. Staining Procedure:

1. Induce apoptosis in cells (1×10^6 /ml) by desired method. Concurrently incubate a control culture *without* induction. An additional negative control can be prepared by adding the caspase inhibitor Z-VAD-FMK at 1 µl/ml to an induced culture to inhibit caspase activation.
2. Aliquot 300 µl each of the induced and control cultures into eppendorf tubes.
3. Add 1 µl of FITC-IETD-FMK into each tube and incubate for 0.5 - 1 hour at 37°C incubator with 5 % CO₂.
4. Centrifuge cells at 3000 rpm for 5 minutes and remove supernatant.
5. Resuspend cells in 0.5 ml of Wash Buffer, and centrifuge again.
6. Repeat Step 5.
Proceed to B, C, or D depending on methods of analysis.

B. Quantification by Flow Cytometry:

For flow cytometric analysis, resuspend cells in 300 µl of Wash buffer. Put samples on ice. Analyzing samples by flow cytometry using the FL-1 channel.

C. Detection by Fluorescence Microscopy:

For fluorescence microscopic analysis, resuspend cells in 100 μ l Wash buffer. Add one drop of the cell suspension onto a microslide and cover with a coverslip. Observe cells under a fluorescence microscope using FITC filter. Caspase positive cells appear to have brighter green signals, whereas caspase-8 negative control cells show much weaker signal.

D. Analysis by Fluorescence Plate Reader:

For analysis with fluorescence plate reader, resuspend cells in 100 μ l Wash Buffer and then transfer the cell suspension to each well of the black microtiter plate. Measure the fluorescence intensity at Ex/Em = 485/535 nm. For control, use wells containing unlabeled cells.

IV. GENERAL TROUBLESHOOTING GUIDE

Problems	Cause	Solution
High background	<ul style="list-style-type: none"> • Cell density is higher than recommended • Cells were not washed well with wash buffer after staining • Cells were Incubated for extended period of time • Use of extremely confluent cells • Cells were contaminated 	<ul style="list-style-type: none"> • Refer to datasheet and use the suggested cell number • Use the wash buffer provided, and as instructed in the datasheet • Refer to datasheets for proper incubation time • Perform assay when cells are at 70-95% confluency • Check for bacteria/ yeast/ mycoplasma contamination
Lower signal level	<ul style="list-style-type: none"> • Cells did not initiate apoptosis • Very few cells were used for analysis • Incorrect setting of the equipment or wavelength used to read samples • Use of expired kit or improperly stored reagents 	<ul style="list-style-type: none"> • Determine the optimal time and dose for apoptosis induction (time-course experiment) • Refer to data sheet for appropriate cell number • Refer to datasheet and use the recommended filter setting • Always check the expiry date and store the components appropriately
Erratic results	<ul style="list-style-type: none"> • Old (unhealthy) cells used • Adherent cells were dislodged and washed away prior to assaying • Incorrect incubation times or temperatures • Incorrect volumes used 	<ul style="list-style-type: none"> • Seed healthy cells and make sure cells are healthy prior to induction of apoptosis • Collect all cells (both attached and dislodged) after induction for accurate results • Refer to datasheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
<p>Note: The most probable cause is listed under each section. Causes may overlap with other sections.</p>		

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