RayBio® CaspGLOW™ Fluorescein Active Caspase-8 Staining Kit

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RayBio® Caspase-8 Fluorometric Assay Kit Protocol

(Cat#: 68FLS-Casp8-S)

RayBiotech, Inc.

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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 x 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 % CO_2.
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 µl 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 µl 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	• Cell density is higher than recommended	• Refer to datasheet and use the suggested cell number
	• Cells were not washed well with wash buffer after staining	• Use the wash buffer provided, and as instructed in the datasheet
	• Cells were Incubated for extended period of time	• Refer to datasheets for proper incubation time
	• Use of extremely confluent cells	• Perform assay when cells are at 70-95% confluency
	• Cells were contaminated	• Check for bacteria/ yeast/ mycoplasma contamination
Lower signal level	• Cells did not initiate apoptosis	• Determine the optimal time and dose for apoptosis induction (time-course experiment)
	• Very few cells were used for analysis	• Refer to data sheet for appropriate cell number
	• Incorrect setting of the equipment or wavelength 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	• Old (unhealthy) cells used	• Seed healthy cells and make sure cells are healthy prior to induction of apoptosis
	• Adherent cells were dislodged and washed away prior to assaying	• Collect all cells (both attached and dislodged) after induction for accurate results
	• Incorrect incubation times or temperatures	• Refer to datasheet & verify correct incubation times and temperatures
	• Incorrect volumes used	• Use calibrated pipettes and aliquot correctly
Note: The most probable cause is listed under each section. Causes may overlap with other sections.		

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