# RayBio® Caspace-12 Fluorometric Assay Kit

**User Manual Version 1.0** 

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RayBio<sup>®</sup> Caspace-12 Fluorometric Assay Kit Protocol

(Cat#: 68FL-Casp12-S)



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## RayBio® Caspase-12 Fluorometric Assay Kit

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### I. INTRODUCTION

Caspase family of proteases are the central mediators of apoptosis in mammalian cells. The **Caspase-12 Fluorometric Assay Kit** provides a simple and convenient means for assaying the activity of caspases that recognize the sequence ATAD. The assay is based on detection of cleavage of substrate ATAD-AFC (AFC: 7-amino-4-trifluoromethyl coumarin). ATAD-AFC emits blue light ( $\lambda$ max = 400 nm); upon cleavage of the substrate by caspase-12 or related caspases, free AFC emits a yellow-green fluorescence ( $\lambda$ max = 505 nm), which can be quantified using a fluorometer or a fluorecence microtiter plate reader. Comparison of the fluorescence of AFC from an apoptotic sample with an uninduced control allows determination of the fold increase in caspase-12 activity.

#### II. KIT CONTENTS

Components	68FL-Casp12- S25	68FL-Casp12-S100	Part Number
	25 assays	100 assays	
Cell Lysis Buffer	25 ml	100 ml	Part A
2X Reaction Buffer	2 ml	4 x 2 ml	Part B
ATAD-AFC (1 mM)	125 μΙ	0.5 ml	Part C
DTT (1 M)	100 μΙ	0.4 ml	Part D

## **III. ASSAY PROTOCOL**

#### A. General Considerations

- Aliquot enough 2X Reaction Buffer for the number of assays to be performed. Add DTT to the 2X Reaction Buffer immediately before use (10 mM final concentration: add 10 µl of 1.0 M DTT stock per 1 ml of 2X Reaction Buffer).
- After thawing, store the Cell Lysis Buffer and 2X Reaction Buffer at 4°C.
- Protect ATAD-AFC from light.

## **B.** Assay Procedure

- 1. Induce apoptosis in cells by desired method. Concurrently incubate a control culture *without* induction.
- 2. Count cells and pellet 2-5 x  $10^{\circ}$  cells or use  $100\text{-}300~\mu g$  cell lysates if protein concentration has been measured.
- 3. Resuspend cells in 50 µl of chilled Cell Lysis Buffer. Incubate on ice for 10 min.
- 4. Add 50 µl of 2X Reaction Buffer (containing 10 mM DTT) to each sample.

- 5. Add 5  $\mu$ l of the ATAD-AFC substrate (50  $\mu$ M final concentration) and incubate at 37 $^{\circ}$  C for 1-2 hour.
- 6. Read samples in a fluorometer equipped with a 400-nm excitation filter and 505-nm emission filter. For a plate-reading set-up, transfer the samples to a 96-well plate. You may also perform the entire assay directly in a 96-well plate. Fold-increase in caspase-12 activity can be determined by comparing these results with the level of the uninduced control.

#### IV. STORAGE AND STABILITY

• Store kit at -20° C (Store Cell Lysis Buffer & 2X Reaction Buffer at 4° C after opening). All reagents are stable for 6 months from date of receipt under proper storage conditions.

### IV. GENERAL TROUBLESHOOTING GUIDE

Experiment was not performed at optimal time after apoptosis induction	Problems	Cause	Solution
optimal time after apoptosis induction  Plate read at incorrect wavelength  Old DTI used  Increased amount of cell lysate used  Increased amounts of components added due to incorrect pipetting  Incubation of cell samples for extended periods  Use of expired kit or improperly stored reagents  Contaminated cells  Colls did not initiate apoptosis  Use of samples stored for a long time  Incorrect setting of the equipment used to read samples  Allowing the reagents to sit for extended times on ice  Samples with erratic readings  Samples with erratic  Cell/ tissue samples were not completely homogenized  Samples were not completely homogenized  Samples coll interfering substance in the sample  Unanticipated results  Unanticipated results  Presence of interfering substances  Improperly thawed components  Incorrect toulmes used  Neesured at incorrect wavelength  All bubbles formed in the well/tube  - Check the wavelength listed in the datasheet and the filter setting of the lock the wavelength is efter to datasheet and use the suggested cell number to prepare lysates  - Use calibrated pipettes  - Refer to datasheet and use the suggested cell number to prepare lysates  - Use calibrated pipettes  - Always scheck the expiry date and store the individual components appropriately  - Check for bacteria/ yeast/ mycoplasma contamination  - Determine the time-point for initiation of apoptosis after induction (time-course experiment)  - Refer to datasheet and use the suggested cell number to prepare lysates  - Neefer to datasheet and incubate for exact times  - Always scheck the expiry date and store the individual components appropriately  - Check for bacteria/ yeast/ mycoplasma contamination  - Determine the time-point for initiation of apoptosis after induction (time-course experiment)  - Refer to datasheet and use the respiry date and store and use within one month for the assay  - Refer to datasheet and incubate for exact times  - Always chaw and prepare fresh reaction mix before use  - Mere to datasheet and use the respiry	Assay not working	Cells did not lyse completely	
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• Air bubbles formed in the well/tube • Pipette gently against the wall of the well/tubes		· ·	•
			1
		Air bubbles formed in the well/tube	
		Substituting reagents from older kits/	Use fresh components from the same kit
lots			
• Use of a different 96-well plate • Fluorescence: Black plates; Absorbance: Clear plates  Note: The most probable cause is listed under each section. Causes may overlap with other sections.		•	

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