

RayBio[®] Caspase-Family Fluorometric Substrate Set Plus

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RayBio[®] Caspase-Family Fluorometric Substrate
Set Plus Protocol

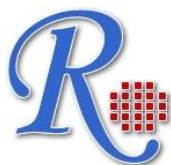
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RayBio® Caspase-Family Fluorometric Substrate Set Plus

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I. INTRODUCTION

Storage Conditions: Store at -20C

Shelf Life: 6-12 months under proper storage conditions

II. KIT CONTENTS: each of the following substrates is dissolved in DMSO

Concentration	Description	Volume	Part Number
1 mM	Caspase-1 Substrate, Ac-YVAD-AFC	125 μ l	Part A
1 mM	Caspase-2 Substrate, Ac-VDVAD-AFC	125 μ l	Part B
1 mM	Caspase-3 Substrate, Ac-DEVD-AFC	125 μ l	Part C
1 mM	Caspase-5 Substrate, Ac-WEHD-AFC	125 μ l	Part D
1 mM	Caspase-6 Substrate, Ac-VEID-AFC	125 μ l	Part E
1 mM	Caspase-8 Substrate, Ac-IETD-AFC	125 μ l	Part F
1 mM	Caspase-9 Substrate, Ac-LEHD-AFC	125 μ l	Part G
N/A	Cell Lysis Buffer	100 ml	Part H
N/A	2X Reaction Buffer	20 ml	Part I
1 M	DTT	0.4 ml	Part J

III. ASSAY PROCEDURE

1. Induce apoptosis in cells by desired method. Concurrently incubate a control culture *without* induction.
2. Count cells and pellet $1-5 \times 10^6$ cells or use 50-200 μ g cell lysates if protein concentration has been measured.
3. Resuspend cells in 50 μ l of chilled Cell Lysis Buffer.
4. Incubate cells on ice for 10 minutes.
5. Add 50 μ l of 2X Reaction Buffer and 1 μ l DTT to each sample.
6. Add 5 μ l of the 1 mM AFC conjugated substrates (50 μ M final conc.) into each tube individually and incubate at 37°C for 1-2 hour.
7. 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 perform the entire assay directly in a 96-well plate.

Fold-increase in caspase activity can be determined by comparing these results with the level of the uninduced control.

IV. GENERAL TROUBLESHOOTING GUIDE

Problems	Cause	Solution
Assay not working	<ul style="list-style-type: none"> • Cells did not lyse completely • Experiment was not performed at optimal time after apoptosis induction • Plate read at incorrect wavelength • Old DTT used 	<ul style="list-style-type: none"> • Resuspend the cell pellet in the lysis buffer and incubate as described in the datasheet • Perform a time-course induction experiment for apoptosis • Check the wavelength listed in the datasheet and the filter settings of the instrument • Always use freshly thawed DTT in the cell lysis buffer
High Background	<ul style="list-style-type: none"> • 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 	<ul style="list-style-type: none"> • Refer to datasheet and use the suggested cell number to prepare lysates • Use calibrated pipettes • Refer to datasheet and incubate for exact times • Always check the expiry date and store the individual components appropriately • Check for bacteria/ yeast/ mycoplasma contamination
Lower signal levels	<ul style="list-style-type: none"> • Cells did not initiate apoptosis • Very few cells used for analysis • 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 	<ul style="list-style-type: none"> • Determine the time-point for initiation of apoptosis after induction (time-course experiment) • Refer to datasheet for appropriate cell number • Use fresh samples or aliquot and store and use within one month for the assay • Refer to datasheet and use the recommended filter setting • Always thaw and prepare fresh reaction mix before use
Samples with erratic readings	<ul style="list-style-type: none"> • Uneven number of cells seeded in the wells • Samples prepared in a different buffer • Adherent cells dislodged and lost at the time of experiment • Cell/ tissue samples were not completely homogenized • Samples used after multiple freeze-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples 	<ul style="list-style-type: none"> • Seed only equal number of healthy cells (correct passage number) • Use the cell lysis buffer provided in the kit • Perform experiment gently and in duplicates/triplicates; apoptotic cells may become floaters • Use Dounce homogenizer (increase the number of strokes); observe efficiency of lysis under microscope • Aliquot and freeze samples, if needed to use multiple times • Troubleshoot as needed • Use fresh samples or store at correct temperatures until use
Unanticipated results	<ul style="list-style-type: none"> • Measured at incorrect wavelength • Cell samples contain interfering substances 	<ul style="list-style-type: none"> • Check the equipment and the filter setting • Troubleshoot if it interferes with the kit (run proper controls)
General issues	<ul style="list-style-type: none"> • Improperly thawed components • Incorrect incubation times or temperatures • Incorrect volumes used • Air bubbles formed in the well/tube • Substituting reagents from older kits/ lots • Use of a different 96-well plate 	<ul style="list-style-type: none"> • Thaw all components completely and mix gently before use • Refer to datasheet & verify the correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly • Pipette gently against the wall of the well/tubes • Use fresh components from the same kit • 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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