

Caspase Fluorometric Substrate Set II Plus



Instruction Manual

Catalog Number	PK-CA577-K137																																							
Description	Contains ready-to-use caspase-1/-2/-3/-4/-5/-6/-8/-9/-10 AFC-labeled substrates and all buffers for performing caspase assay. All substrates are provided in liquid ready-to-use form.																																							
Kit Components	<table border="1"> <thead> <tr> <th>Concentration</th> <th>Description</th> <th>Volume</th> </tr> </thead> <tbody> <tr> <td>1 mM</td> <td>Caspase-1 Substrate, Ac-YVAD-AFC</td> <td>125 µl</td> </tr> <tr> <td>1 mM</td> <td>Caspase-2 Substrate, Ac-VDVAD-AFC</td> <td>125 µl</td> </tr> <tr> <td>1 mM</td> <td>Caspase-3/7 Substrate, Ac-DEVD-AFC</td> <td>125 µl</td> </tr> <tr> <td>1 mM</td> <td>Caspase-4 Substrate, Ac-LEVD-AFC</td> <td>125 µl</td> </tr> <tr> <td>1 mM</td> <td>Caspase-5 Substrate, Ac-WEHD-AFC</td> <td>125 µl</td> </tr> <tr> <td>1 mM</td> <td>Caspase-6 Substrate, Ac-VEID-AFC</td> <td>125 µl</td> </tr> <tr> <td>1 mM</td> <td>Caspase-8 Substrate, Ac-IETD-AFC</td> <td>125 µl</td> </tr> <tr> <td>1 mM</td> <td>Caspase-9 Substrate, Ac-LEHD-AFC</td> <td>125 µl</td> </tr> <tr> <td>1 mM</td> <td>Caspase-10 Substrate, Ac-AEVD-AFC</td> <td>125 µl</td> </tr> <tr> <td>N/A</td> <td>Cell Lysis Buffer</td> <td>100 ml</td> </tr> <tr> <td>N/A</td> <td>2X Reaction Buffer</td> <td>20 ml</td> </tr> <tr> <td>1 M</td> <td>DTT</td> <td>0.4 ml</td> </tr> </tbody> </table>	Concentration	Description	Volume	1 mM	Caspase-1 Substrate, Ac-YVAD-AFC	125 µl	1 mM	Caspase-2 Substrate, Ac-VDVAD-AFC	125 µl	1 mM	Caspase-3/7 Substrate, Ac-DEVD-AFC	125 µl	1 mM	Caspase-4 Substrate, Ac-LEVD-AFC	125 µl	1 mM	Caspase-5 Substrate, Ac-WEHD-AFC	125 µl	1 mM	Caspase-6 Substrate, Ac-VEID-AFC	125 µl	1 mM	Caspase-8 Substrate, Ac-IETD-AFC	125 µl	1 mM	Caspase-9 Substrate, Ac-LEHD-AFC	125 µl	1 mM	Caspase-10 Substrate, Ac-AEVD-AFC	125 µl	N/A	Cell Lysis Buffer	100 ml	N/A	2X Reaction Buffer	20 ml	1 M	DTT	0.4 ml
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Quantity	9 x 25 assays																																							
Sequence / Molecular Weight / Molecular Formula	see data sheets of individual caspase substrates																																							
Purity	see data sheets of individual caspase substrates																																							
Appearance / Formulation / Solubility	Solution in DMSO																																							
Storage & Stability	Store at -20°C. Stable for 6-12 months under proper storage conditions.																																							
Applications	<p>1. Induce apoptosis in cells by desired method. Concurrently incubate a control culture without induction.</p> <p>2. Count cells and pellet $1-5 \times 10^6$ cells or use 50-200 µg cell lysates if protein concentration has been measured.</p> <p>3. Resuspend cells in 50 µl of chilled Cell Lysis Buffer (Cat.# PK-CA577-1067-100).</p> <p>4. Incubate cells on ice for 10 minutes.</p> <p>5. Add 50 µl of 2X Reaction Buffer and 1 µl DTT to each sample.</p> <p>6. Add 5 µl of the 1 mM AFC conjugated substrates (50 µM final conc.) into each tube individually and incubate at 37°C for 1-2 hour.</p> <p>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.</p> <p>Fold-increase in caspase activity can be determined by comparing these results with the level of the uninduced control.</p>																																							
References	NA																																							
Caution	NA																																							

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