**Cell Meter™ Live Cell Caspase 3/7 Binding Assay Kit**  
*Red Fluorescence*

<table>
<thead>
<tr>
<th>Ordering Information</th>
<th>Storage Conditions</th>
<th>Instrument Platform</th>
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</table>
| Product Number: 20101 (25 assays) | Keep in freezer  
Avoid light | Fluorescent microscopy, flow cytometer,  
and fluorescent microplate reader |

**Introduction**

Our Cell Meter™ live cell caspases activity assay kits are based on fluorescent inhibitors of caspases. These inhibitors are cell permeable and non-cytotoxic. Once inside the cell, the caspase inhibitors bind covalently to the active caspases. The activation of caspase 3/7 is important for the initiation of apoptosis. It has been proven that caspase 3/7 has substrate selectivity for the peptide sequence Asp-Glu-Val-Asp (DEVD). This kit uses TF3-DEVD-FMK as a fluorescent indicator for caspase 3/7 activity. TF3-DEVD-FMK irreversibly binds to activated caspase 3/7 in apoptotic cells. Once bound to caspase 3/7, the fluorescent reagent is retained inside the cell. The binding event inhibits caspase 3/7 but will not stop apoptosis from proceeding.

There are a variety of parameters that can be used for monitoring cell apoptosis. This Cell Meter™ Live Cell Caspase 3/7 Activity Assay Kit is designed to detect cell apoptosis by measuring caspase 3/7 activation in live cells. It is used for the quantification of activated caspase 3/7 activities in apoptotic cells, or for screening caspase 3/7 inhibitors. TF3-DEVD-FMK, the red label reagent, allows for direct detection of activated caspase 3/7 in apoptotic cells by fluorescence microscopy, flow cytometer, or fluorescent microplate reader. The kit provides all the essential components with an optimized assay protocol.

**Kit Components**

<table>
<thead>
<tr>
<th>Components</th>
<th>Amount</th>
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<tbody>
<tr>
<td>Component A: TF3-DEVD-FMK</td>
<td>1 vial</td>
</tr>
<tr>
<td>Component B: Washing Buffer</td>
<td>1 bottle (100 mL)</td>
</tr>
<tr>
<td>Component C: 500X Nuclear Green™ DCS1</td>
<td>1 vial (100 µL)</td>
</tr>
<tr>
<td>Component D: 500X Hoechst</td>
<td>1 vial (100 µL)</td>
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**Assay Protocol for Detached Cells**

**Brief Summary**

1. Prepare cells with test compounds at a density of $5 \times 10^5$ to $2 \times 10^6$ cells/mL  
   Add TF3-DEVD-FMK into cell solution at 1:150 ratio  
   Incubate at room temperature for 1 hour  
   Pellet the cells, wash and resuspend the cells with buffer or growth medium  
   Analyze the cells at Ex/Em = 550/595 nm

*Note: Thaw all the components at room temperature before use.*
1) Treating Jurkat cells with 2 μg/ml camptothecin for 3 hours.
2) Treating Jurkat cells with 1 μM staurosporine for 3 hours.
3) Treating HL-60 cells with 4 μg/ml camptothecin for 4 hours.
4) Treating HL-60 cells with 1 μM staurosporine for 4 hours.

*Note: Each cell line should be evaluated on an individual basis to determine the optimal cell density for apoptosis induction.*

2. Make 150X TF3-DEVD-FMK DMSO stock solution by adding 50 μL of DMSO to the vial of TF3-DEVD-FMK (Component A). Add 150 X TF3-DEVD-FMK into the cell solution at a 1:150 ratio, and incubate the cells in a 37°C, 5% CO₂ incubator for 1 hour.

*Note 1: The cells can be concentrated up to ~ 5 X 10⁶ cells/mL for TF3-DEVD-FMK labeling. The unused 150X TF3-DEVD-FMK DMSO stock solution should be divided as single use aliquot and stored at -20°C.*

*Note 2: For adherent cells, gently lift the cells with 0.5 mM EDTA to keep the cells intact, and wash the cells once with serum-containing media prior to incubation with TF3-DEVD-FMK.*

*Note 3: The appropriate incubation time depends on the individual cell type and cell concentration used. Optimize the incubation time for each experiment.*

3. Spin down the cells at ~ 200g for 5 minutes, and wash cells with 1 mL washing buffer (Component B) twice. Resuspend the cells in desired amount of washing buffer.

*Note 1: TF3-DEVD-FMK is fluorescent, thus it is important to wash out any unbound reagent to eliminate the background.*

*Note 2: For detached cells, the concentration of cells should be adjusted to 2-5 X 10⁵ cells/100 μL aliquot per microtiter plate well for use in step 5.*

4. If desired, label the cells with a DNA stain (such as Nuclear Green™ DCS1 for dead cells, or Hoechst for whole population of the cell nucleus stain).

5. Monitor the fluorescence intensity by fluorescence microscopy, flow cytometer, or fluorescent microplate reader at Ex/Em = 550/595 nm (for Nuclear Green™ DCS1, Ex/Em = 490/525 nm, for Hoechst dyes, Ex/Em = 350/461 nm)

5.1 For flow cytometry, monitor the fluorescence intensity using the channel with Ex/Em = 550/595 nm (FL1 channel for Nuclear Green™ DCS1 staining). Gate on the cells of interest, excluding debris.

5.2 For fluorescence microscopy and fluorescent microplate reader. Place 100 μL of the cell suspensions into each of wells of a 96-well black microtiter plate.

*Note: If it is necessary to equilibrate the cell concentrations, adjust the suspension volume for the induced cells to approximate the cell density of the non-induced population. This adjustment step is optional if your cell treatment does not result in a dramatic loss in stimulated cell population numbers.*

5.3 Observe cells under a fluorescence microscope using TRITC channel (FITC channel for Nuclear Green™ DCS1 staining, DAPI channel for Hoechst staining).

5.4 Monitor the fluorescence intensity using Ex/Em = 550/595 nm (cut off at 570 nm) bottom read mode using a fluorescent microplate reader.
Data Analysis

1. 96-Well Fluorescence Plate Reader Sample Data:

Figure 1. TF3-DEVD-FMK fluorometric detection of active caspases 3/7 using Kit #20101 in Jurkat cells. The cells were treated with 1 μM staurosporine for 3 hours (Red) while untreated cells were used as a control (Blue). Cells were incubated with TF3-DEVD-FMK for 1 hour at 37°C. The Fluorescent intensity (300, 000 cells/100 μL/well) was measured at Ex/Em = 550/595 nm (cut off at 515 nm) with a FlexStation microplate reader using bottom read mode.

2. Fluorescence Microscopy Sample Data:

Figure 2. The Fluorescent Microscopy showing the increase in TF3-DEVD-FMK fluorescence intensity with the addition of 1 μM Staurosporin in Jurkat cells. Cells were incubated with TF3-DEVD-FMK for 1 hour at 37°C. The fluorescent intensity of the cells (200,000 cells/100 μL per well) was viewed under a fluorescence microscope with a TRITC channel.

References


Warning: This kit is only sold to end users. Neither resale nor transfer to a third party is allowed without written permission from AAT Bioquest®. Chemical analysis of the kit components is strictly prohibited. Please call us at 408-733-1055 or e-mail us at info@aatbio.com if you have any questions.