Product Information

Cathepsin D Activity Fluorometric Assay Kit

I. Kit Contents:

<table>
<thead>
<tr>
<th>Component</th>
<th>K2154-100 100 assays</th>
<th>Cap Color</th>
<th>Part Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD Cell Lysis Buffer</td>
<td>25 ml</td>
<td>WM</td>
<td>K2154-C-1</td>
</tr>
<tr>
<td>CD Reaction Buffer</td>
<td>5 ml</td>
<td>NM</td>
<td>K2154-C-2</td>
</tr>
<tr>
<td>CD Substrate (1mM)</td>
<td>0.2 ml</td>
<td>Brown</td>
<td>K2154-C-3</td>
</tr>
</tbody>
</table>

II. Introduction:

Apoptosis is often mediated by the traditional caspase-mediated cleavage cascade. In addition, alternative proteolytic enzymes such as the lysosomal cathepsin proteases can also initiate or propagate proapoptotic signals. Cathepsins are lysosomal proteases that play an important role in mammalian cellular turnover such as bone resorption. Cathepsins are often used as sensitive markers in a variety of toxicological investigations.

Cathepsin D is a lysosomal aspartyl protease belonging to the peptidase A1 family and is used as a marker of breast cancer tumor.

The Cathepsin D Activity Fluorometric Assay Kit provides a sensitive, simple and convenient way for detection of cathepsin D activity based on fluorometric method. The assay utilizes the preferred cathepsin-D substrate sequence GKPILFFRLK(Dnp)-D-R-NH2 labeled with MCA. While cleavage of the synthetic substrate by cathepsin-D in cell lysates or other samples, the released fluorescence (Ex/Em = 328/460 nm) can be easily quantified using a fluorescence microtiter plate reader or a fluorometer.

III. Cathepsin D Assay Protocol:

1. Collect cells (1 x 10^6) by centrifugation.
2. Lyse cells in 200 µl of chilled CD Cell Lysis Buffer. Incubate cells on ice for 10 min.
3. Centrifuge for 5 min at top speed. Transfer the clear cell lysate into a labeled new tube.
4. Add 5 - 50 µl of the cell lysate (or ~ 1 - 10 ng of purified Cathepsin D protein samples) into each well in a 96-well plate. Bring the total volume to 50 µl with CD Cell Lysis Buffer.
5. Prepare a master assay mix, for each assay:
   - Reaction Buffer  50 µl
   - Substrate        2 µl
6. Mix the master assay mix. Add 52 µl of the master assay mix into each assay wells. Mix well. Incubate at 37°C for 1 - 2 hour.
7. Read samples in a fluorometer equipped with a 328-nm excitation filter and 460-nm emission filter.

Cathepsin D activity can be expressed by the relative fluorescence units (RFU) per million cells, or RFU per microgram protein of your sample, or RFU fold increase of treated samples vs the untreated control or the negative control sample.

IV. Storage and Stability:

Store kit at -20°C (Store CD Cell Lysis Buffer and CD Reaction Buffer at 4°C after opening). Protect CD Substrate from light. All reagents are stable for 6 months under proper storage conditions.
### General Troubleshooting Guide for Cathepsin Fluorometric Kits:

<table>
<thead>
<tr>
<th>Problems</th>
<th>Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay not working</td>
<td>• Cells did not lyse completely</td>
<td>• Resuspend the cell pellet in the lysis buffer and incubate as described in the datasheet</td>
</tr>
<tr>
<td></td>
<td>• Experiment was not performed at optimal time after apoptosis induction</td>
<td>• Perform a time-course induction experiment for apoptosis</td>
</tr>
<tr>
<td></td>
<td>• Plate read at incorrect wavelength</td>
<td>• Check the wavelength listed in the datasheet and the filter settings of the instrument</td>
</tr>
<tr>
<td>High Background</td>
<td>• Increased amount of cell lysate used</td>
<td>• Refer to datasheet and use the suggested cell number to prepare lysates</td>
</tr>
<tr>
<td></td>
<td>• Increased amounts of components added due to incorrect pipetting</td>
<td>• Use calibrated pipettes</td>
</tr>
<tr>
<td></td>
<td>• Incubation of cell samples for extended periods</td>
<td>• Refer to datasheet and incubate for exact times</td>
</tr>
<tr>
<td></td>
<td>• Use of expired kit or improperly stored reagents</td>
<td>• Always check the expiry date and store the individual components appropriately</td>
</tr>
<tr>
<td></td>
<td>• Contaminated cells</td>
<td>• Check for bacteria/ yeast/ mycoplasma contamination</td>
</tr>
<tr>
<td>Lower signal levels</td>
<td>• Cells did not initiate apoptosis</td>
<td>• Determine the time-point for initiation of apoptosis after induction (time-course experiment)</td>
</tr>
<tr>
<td></td>
<td>• Very few cells used for analysis</td>
<td>• Refer to datasheet for appropriate cell number</td>
</tr>
<tr>
<td></td>
<td>• Use of samples stored for a long time</td>
<td>• Use fresh samples or aliquot and store and use within one month for the assay</td>
</tr>
<tr>
<td></td>
<td>• Incorrect setting of the equipment used to read samples</td>
<td>• Refer to datasheet and use the recommended filter setting</td>
</tr>
<tr>
<td></td>
<td>• Allowing the reagents to sit for extended times on ice</td>
<td>• Always thaw and prepare fresh reaction mix before use</td>
</tr>
<tr>
<td>Samples with erratic readings</td>
<td>• Uneven number of cells seeded in the wells</td>
<td>• Seed only equal number of healthy cells (correct passage number)</td>
</tr>
<tr>
<td></td>
<td>• Samples prepared in a different buffer</td>
<td>• Use the cell lysis buffer provided in the kit</td>
</tr>
<tr>
<td></td>
<td>• Adherent cells dislodged and lost at the time of experiment</td>
<td>• Perform experiment gently and in duplicates/triplicates; apoptotic cells may become floaters</td>
</tr>
<tr>
<td></td>
<td>• Cell/ tissue samples were not completely homogenized</td>
<td>• Use Dounce homogenizer (increase the number of strokes); observe efficiency of lysis under microscope</td>
</tr>
<tr>
<td></td>
<td>• Samples used after multiple freeze-thaw cycles</td>
<td>• Aliquot and freeze samples, if needed to use multiple times</td>
</tr>
<tr>
<td></td>
<td>• Presence of interfering substance in the sample</td>
<td>• Troubleshoot as needed</td>
</tr>
<tr>
<td></td>
<td>• Use of old or inappropriately stored samples</td>
<td>• Use fresh samples or store at correct temperatures until use</td>
</tr>
<tr>
<td>Unanticipated results</td>
<td>• Measured at incorrect wavelength</td>
<td>• Check the equipment and the filter setting</td>
</tr>
<tr>
<td></td>
<td>• Cell samples contain interfering substances</td>
<td>• Troubleshoot if it interferes with the kit (run proper controls)</td>
</tr>
<tr>
<td>General issues</td>
<td>• Improperly thawed components</td>
<td>• Thaw all components completely and mix gently before use</td>
</tr>
<tr>
<td></td>
<td>• Incorrect incubation times or temperatures</td>
<td>• Refer to datasheet &amp; verify the correct incubation times and temperatures</td>
</tr>
<tr>
<td></td>
<td>• Incorrect volumes used</td>
<td>• Use calibrated pipettes and aliquot correctly</td>
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<tr>
<td></td>
<td>• Air bubbles formed in the well/tube</td>
<td>• Pipette gently against the wall of the well/tubes</td>
</tr>
<tr>
<td></td>
<td>• Substituting reagents from older kits/ lots</td>
<td>• Use fresh components from the same kit</td>
</tr>
<tr>
<td></td>
<td>• Use of a different 96-well plate</td>
<td>• Fluorescence: Black plates; Absorbance: Clear plates</td>
</tr>
</tbody>
</table>

Note: The most probable cause is listed under each section. Causes may overlap with other sections.
For research use only! Not to be used in humans.

Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

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