# Product Information

## Cathepsin D Inhibitor Screening Kit (Fluorometric)

### I. Kit Contents:

| Component | K2156-100<br>100 assays | Cap Color | Part Number |
|---|---|---|---|
| CD Reaction Buffer | 10 ml | WM | K2156-C-1 |
| CD Substrate (1 mM) | 0.2 ml | Brown | K2156-C-2 |
| Cathepsin D, human (lyophilized) | 1 vial | Green | K2156-C-3 |
| Pepstatin A (1 mM in DMSO) | 20 µl | Blue | A2571 |

### 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 Inhibitor Screening Kit (Fluorometric) provides a simple, fast and convenient way for screening of cathepsin D inhibitors 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, the released fluorescence (Ex/Em = 328/460 nm) can be easily quantified using a fluorecence microtiter plate reader or a fluorometer. The relative efficacy of the test inhibitor is compared to the positive control inhibitor Pepstatin A (IC50 < 0.1 nM). The kit is suitable for high throughput screening (HTS) and can be assayed in 96-well plate.

### III. Reagent Preparation: (Please read entire protocol before proceeding)

1. Cathepsin D: Reconstitute with 1 ml of $dH_2O$, aliquot and store at -80℃ Avoid repeated Freeze/Thaw cycles.

2. Pepstatin A: Store at -20℃ To make working solution, take 2 µl of Pepstatin A stock solution and dilute with 798 µl CD Reaction Buffer. Working solution is stable for a day at RT & then should be discarded.

### IV. Cathepsin D Assay Protocol:

1. Prepare Positive Control by mixing 10 µl reconstituted Cathepsin D with 40 µl CD Reaction Buffer.

2. Prepare a Background Control with 50 µl of CD Reaction Buffer alone.

3. Prepare an Inhibitor Reference Control by mixing 10 µl reconstituted Cathepsin D with 10 µl of Pepstatin A working solution and 30 µl CD Reaction Buffer.

4. Prepare Test Inhibitor Samples by mixing 10 µl reconstituted Cathepsin D with 10 µl of the Test Inhibitor and 30 µl CD Reaction Buffer.

5. Pre-incubate Controls and Test Samples at 37℃ for 10 min.

6. Prepare Substrate Mix:

CD Substrate 2 µl

Reaction Buffer 48 µl

Add 50 µl to each well containing Controls and Test Samples. Tap plate gently to mix then incubate at 37℃ for 1 - 2 hour.

7. Read samples in a fluorometer equipped with a 328-nm excitation filter and 460-nm emission filter.

8. Calculation:

% Inhibition = ((RFU Test Inhibitor-RFU Background Control)))/((RFU Positive Control-RFU Background Control)) x 100.

Or

% Inhibition Relative to Pepstatin A = ((RFU Test Sample -RFU Background Control)))/((RFU Negative Control- RFU Background Control)) x 100.

## V. Storage and Stability:

Store kit at -20℃ (Store CD Reaction Buffer at 4℃ after opening). Protect CD Substrate from light. Store Cathepsin D vial at -80℃ All reagents are stable for 6 months under proper storage conditions.

### General Troubleshooting Guide:

| Problems | Cause | Solution |
|---|---|---|
| Assay not working | • Cells did not lyse completely<br>• Experiment was not performed at optimal time after apoptosis induction<br>• Plate read at incorrect wavelength | • Resuspend the cell pellet in the lysis buffer and incubate as described in the datasheet<br>• Perform a time-course induction experiment for apoptosis<br>• Check the wavelength listed in the datasheet and the filter settings of the instrument |
| High Background | • Increased amount of cell lysate used<br>• Increased amounts of components added due to incorrect pipetting<br>• Incubation of cell samples for extended periods<br>• Use of expired kit or improperly stored reagents<br>• Contaminated cells | • Refer to datasheet and use the suggested cell number to prepare lysates<br>• Use calibrated pipettes<br>• Refer to datasheet and incubate for exact times<br>• Always check the expiry date and store the individual components appropriately<br>• Check for bacteria/ yeast/ mycoplasma contamination |
| Lower signal levels | • Cells did not initiate apoptosis<br>• Very few cells used for analysis<br>• Use of samples stored for a long time<br>• Incorrect setting of the equipment used to read samples<br>• Allowing the reagents to sit for extended times on ice | • Determine the time-point for initiation of apoptosis after induction (time-course experiment)<br>• Refer to datasheet for appropriate cell number<br>• Use fresh samples or aliquot and store and use within one month for the assay<br>• Refer to datasheet and use the recommended filter setting<br>• Always thaw and prepare fresh reaction mix before use |
| Samples with erratic readings | • Uneven number of cells seeded in the wells<br>• Samples prepared in a different buffer<br>• Adherent cells dislodged and lost at the time of experiment<br>• Cell/ tissue samples were not completely homogenized<br>• Samples used after multiple freeze-thaw cycles<br>• Presence of interfering substance in the sample<br>• Use of old or inappropriately stored samples | • Seed only equal number of healthy cells (correct passage number)<br>• Use the cell lysis buffer provided in the kit<br>• Perform experiment gently and in duplicates/triplicates; apoptotic cells may become floaters<br>• Use Dounce homogenizer (increase the number of strokes); observe efficiency of lysis under microscope<br>• Aliquot and freeze samples, if needed to use multiple times<br>• Troubleshoot as needed<br>• Use fresh samples or store at correct temperatures until use |

| | | |
|---|---|---|
| Unanticipated results | • Measured at incorrect wavelength<br>• Cell samples contain interfering substances | • Check the equipment and the filter setting<br>• Troubleshoot if it interferes with the kit (run proper controls) |
| General issues | • Improperly thawed components<br>• Incorrect incubation times or temperatures<br>• Incorrect volumes used<br>• Air bubbles formed in the well/tube<br>• Substituting reagents from older kits/ lots<br>• Use of a different 96-well plate | • Thaw all components completely and mix gently before use<br>• Refer to datasheet & verify the correct incubation times and temperatures<br>• Use calibrated pipettes and aliquot correctly<br>• Pipette gently against the wall of the well/tubes<br>• Use fresh components from the same kit<br>• Fluorescence: Black plates; Absorbance: Clear plates |
| 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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