MA-0154

Cathepsin D Activity Assay

(100 wells, Fluorometric, Ex/Em = 328/460 nm, Store at -20°C)

Introduction:

Cathepsin D performs a variety of functions in the cell, including recycling of protein through autophagy, maturation of proteins by cleaving precursor proteins and apoptosis. Cathepsin D has a dual role with respect to apoptosis. On the one hand, under conditions of cellular stress such as DNA damage or oxidative stress, Cathepsin D is released from the lysosomes and activates apoptotic pathways by cleaving pro-apoptotic proteins. On the other hand, Cathepsin D can prevent apoptosis by regulating the stability and activity of anti-apoptotic proteins, inhibiting apoptotic pathways, enhancing cellular survival and tissue protection. AkrivisBio's Cathepsin D Activity Assay is a simple, sensitive means of measuring Cathepsin D activity in a variety of biological samples with a sensitivity able to detect Cathepsin B in 500 cells or less.

Assay Principle:

Cathepsin B cleaves a quenched substrate releasing a highly fluorescent cleavage product with fluorescence at 328nm/460 nm.

Assay Components:

Cell Lysis Buffer	25 ml	WM	MA-0154A
Assay Buffer	5 ml	NM	MA-0154B
Cathepsin D/E Substrate	0.2 ml	Brown	MA-0154C
Cathepsin D Positive Control	lyoph	Green	MA-0154D
Cathepsin E Positive Control	lyoph	Blue	MA-0154E
MCA Standard	25 µl	Yellow	MA-0154F

Storage and Handling Considerations:

Store unopened assay at -20°C.
Cell Lysis Buffer, Assay Buffer: Ready to use as supplied. Bring to room temperature before use. Store at 4°C.
Cathepsin D/E Substrate: Ready to use as supplied. Bring to room temperature before use to melt DMSO. Store at -20°C
Cathepsin D/ Cathepsin E Positive Controls: Reconstitute with 100 µl Reaction Buffer. Aliquot each to 10 µl portions; freeze at -70°C. Keep on ice while in use.
MCA Standard: 4 µM, Ready to use as supplied. Protect from light. Bring to room temperature before using. Store at -20°C

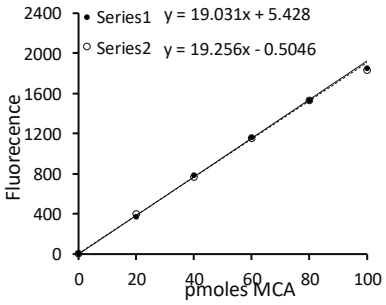
Assay Protocol:

1. Prewarm the plate reader to 37°C
2. **Standard Curve:** Add 0 – 5 – 10 – 15 – 20 – 25 µl to a series of wells of a 96-well plate. Adjust all well volumes to 100 µl with Assay Buffer giving 0 – 20 – 40 – 60 – 80 – 100 pmoles MCA.
3. **Samples:**
   Liquid Samples: Can be added directly to wells of a 96-well plate.
   Cells: Collect cells (1 x 10^6) by centrifugation.
   Lyse cells in 100 µl of chilled Cell Lysis Buffer. Incubate cells on ice for 10 min.
   Tissue: Homogenize 10 mg of tissue in 100 µl of chilled Cell Lysis Buffer
4. **Centrifuge** lysates at 16,000 x g for 5 minutes at 4°C. Transfer the clear cell lysate into fresh tubes.
5. **Transfer** up to 50 µl of supernatant to wells in a 96-well plate. Adjust all sample well volumes to 50 µl with Cell Lysis Buffer.
6. **Positive Controls:** Add 2 – 10 µl of Positive Controls to wells and adjust the volume to 50 µl with Cell Lysis Buffer.
7. Add any inhibitors to be used at this point and allow them to incubate for 10-15 minutes.
8. **Reaction Mix:** Each sample and positive control well (but NOT the Standards wells) will require 50 µl of Reaction Mix. Prepare sufficient material for the number of wells to be run containing:

Reaction Mix	
Reaction Buffer:	48 µl
Cathepsin D/E substrate:	2 µl

   Add 50 µl of the Reaction Mix into each sample and positive control well and mix.
9. Place plate into plate reader and monitor reaction progress at 37°C for 1-2 hours using excitation 328 nm, emission 460 nm.
10. **Typical results:**

	Standard Raw Values		Background Corrected Values	
pmole Standard	RFU			
0	38.708	40.989	0	0
20	410.393	434.580	371.685	393.591
40	823.439	807.722	784.731	766.733
60	1200.316	1187.274	1161.608	1146.285
80	1569.332	1567.764	1530.624	1526.775
100	1886.393	1873.968	1847.685	1832.979



11. **Calculations:** Subtract the zero standard from all standards. Plot the Standard Curve. Determine the slope of the Standard Curve (RFU/pmol). Due to innate curvature of fluorescence data, the highest value can frequently be disregarded in the construction of the linear best fit. Determine the most linear portion for the Cathepsin B reactions. Determine the slope of the linear portion of the reactions (RFU/minute). Divide the enzymatic linear rate by the slope of the standard curve (RFU/min) / (RFU/pmol) to obtain pmol/min or µU of enzyme activity in the well. To convert that to enzyme activity in the original sample:

A. Divide the pmol/min in the well by the volume of sample in µl added to each well = µU of enzyme activity per µl of sample.
B. Multiply the µU of enzyme activity per µl of sample by the total volume of supernatant recovered in step 4 above = total µU of enzyme activity per sample.
C. Divide the total µU of enzyme activity per sample by the mg of initial tissue or # of cells used to derive each sample = µU of enzyme activity per mg of tissue (or per # of cells, etc.)

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