Activity test:

Based on the instructions in the Cathepsin L Activity Fluorometric Assay Kit, we designed the relevant experiments as follows:

1. Add 180ul CL Buffer into 27 wells(A1-A9, B1-B9, C1-C9) in a 96-well plate.
2. Add 0 (A1-C1), 1 (A2-C2), 2 (A3-C3), 3 (A4-C4), 4 (A5-C5), 5 (A6-C6), 6 (A7-C7), 7 (A8-C8), and 8μL (A9-C9) of the 10mM CL Substrate Ac-RR-AFC into plate.
3. Add 20ul cathepsin L protein into each well.
4. Read sample in a fluorometer equipped with a 400-nm excitation filter and 505-nm emission filter. Measure every hour until the fluorescence value remain unchanged.
5. Calculate the initial speed：

substrate(ul)	0	1	2	3	4	5	6	7	8
concentration(mol/L)	0	0.25	0.5	0.75	1	1.25	1.5	1.75	2
V0(RFU/h)	0	15.124	30.851	36.916	40.121	42.075	42.539	43.681	44.01

Michaelis equation represents a velocity equation of the relationship between the initial rate of enzymatic reaction and the concentration of substrate. The formula is as follows

$$v_0 = \frac{V_{max}[S]}{K_m + [S]}$$

V0 represents the initial reaction velocity, Vmax is the reaction rate of enzymes saturated by substrates, and [S] is the concentration of substrate.

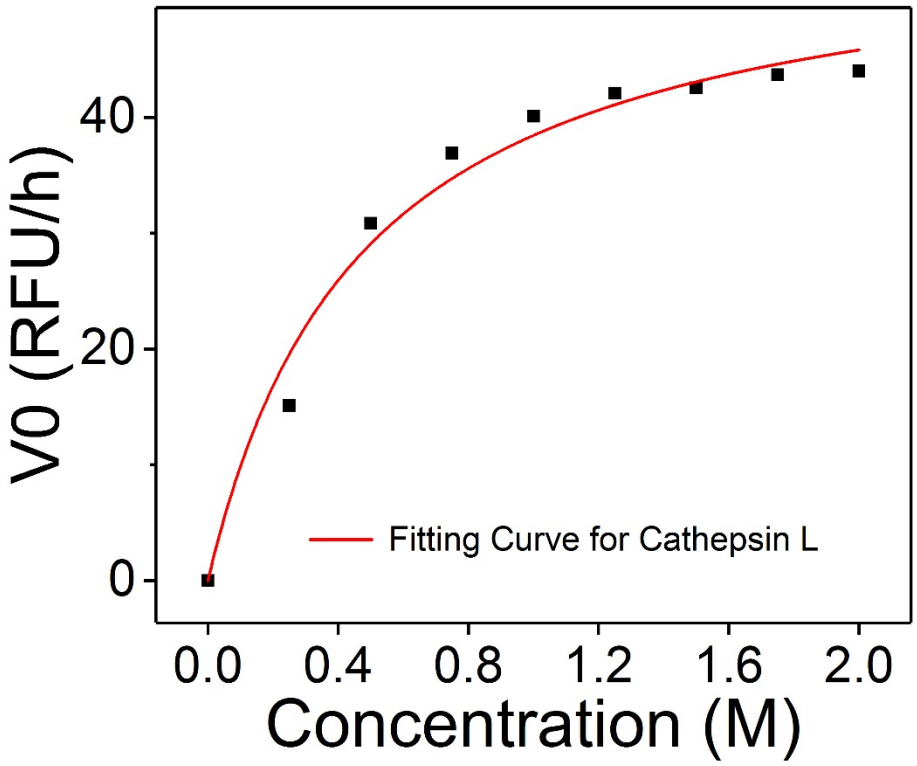
We determined Km and Vmax for our cathepsin L by performing non-linear regression using Michaelis-Menten model as below. The two parameters were:

Vmax=56.71616

Km=0.47541

R^2= 0.98104

Adjusted R^2= 0.97834


V0 (RFU/h)
40
20
0
0.0
0.4
0.8
1.2
1.6
2.0
Concentration (M)
Fitting Curve for Cathepsin L