Activity test:

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

1. Add 180ul CD Reaction Buffer into 15 wells(A1-A4 B1-B4, C1-C4) in a 96-well plate.
2. Add 0 (A1-C1), 2(A2-C2), 4 (A3-C3), 6 (A4-C4), 8 (A5-C5) of the 1mM CD Substrate into plate.
3. Add 20ul cathepsin D protein into each well.
4. Read sample in a fluorometer equipped with a 328-nm excitation filter and 460-nm emission filter. Measure every few seconds. The interval depends on the reaction rate.
5. Calculate the initial speed：

substrate(ul)	0	2	4	6	8
concentration(mol/L)	0	0.5	1	1.5	2
V0(RFU/h)	0	136.63	270.93	386.44	403.17

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.

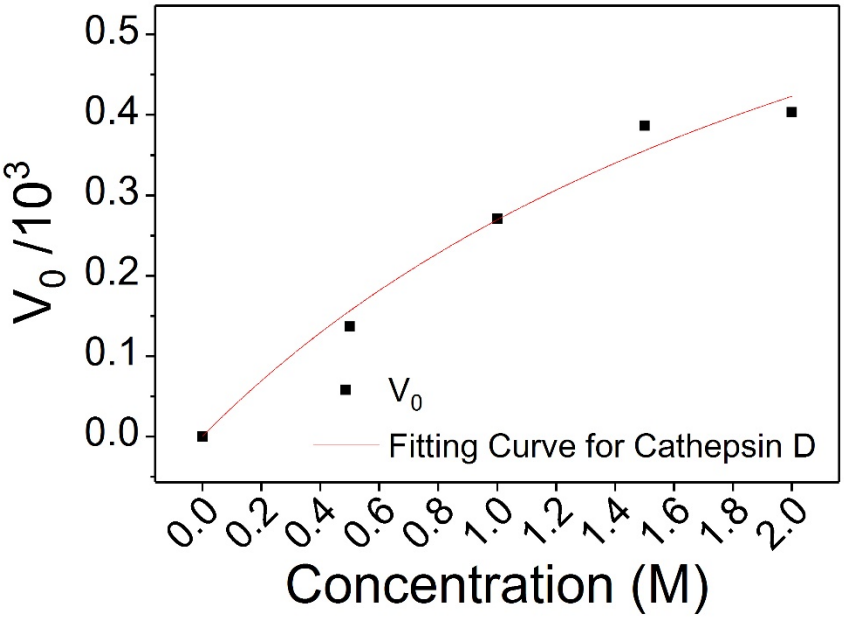
We determined Km and Vmax for our cathepsin B by performing non-linear regression using Michaelis-Menten model as below. The two parameters were:

Vmax=984.68468 ± 310.2332

Km=2.65534 ± 1.30479

R^2= 0.98522

Adjusted R^2= 0.98029


0.5
0.4
0.3
0.2
0.1
0.0
V_0 /10^3
V_0
Fitting Curve for Cathepsin D
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
Concentration (M)