Modelling – Cathepsin L activity test

Aim

BioVision Cathepsin-L activity detection kit is a fluorescence-based detection technology, using AFC (7-amino-4-trifluoromethyl coumarin) labeled Cathepsin-L priority substrate sequence FR. Cell lysates or other samples containing Cathepsin-L can digest FR-AFC and release free AFC, which can be easily quantitatively detected by fluorometer or fluorescence microtitrator reader. This kit can test the activity of our proteins.

Materials

CL Buffer

DTT

CL Substrate Substrate Ac-FR-AFC

Procedure

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.