# Modelling – Cathepsin B activity test

## Aim

The preferential substrate sequence RR of Cathepsin-B labeled with AFC (7-amino-4-trifluoromethyl coumarin). Cell solutes or other samples containing Cathepsin-B can digest RR-AFC and release free AFC. Free AFC can be easily quantified by fluorometer or fluorescent microtitrator plate. To test whether the enzymes expressed by our group are active, we designed a series of enzyme activity experiments

## Materials

CB Reaction Buffer

CB Substrate Ac-RR-AFC

## Procedure

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

1. Add 180ul CB Reaction 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 CB Substrate Ac-RR-AFC into plate.

3. Add 20ul cathepsin B 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.