SOP for Enzymatic Activity Inhibition Assay

This is an example protocol adapted for MMP2 enzymatic activity inhibition. The choice of the technique to measure enzyme inhibition depends on the system being analysed. For an extended version of the protocol, including various considerations, please refer to the corresponding paper to be published in the special issue of *European Biophysics Journal*.

Equipment and materials

Plate reader with fluorescence detection (in this case BioTek Synergy H4 Microplate Reader)

black-wall, clear-bottom 96-well microplates for fluorescence-based assays

tubes

enzyme: matrix metalloproteinase 2 (MMP2)

substrate: Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH_2 (Mca=(7-methoxycoumarin-4-yl)acetyl and Dpa=N-3-(2,4-dinitrophenyl)-L-α,β-diaminopropionyl)

inhibitor: marimastat

DMSO

Assay buffer: 50 mM TRIS (pH 7.5), 150 mM NaCl, 10 mM $CaCl_2$

Protocol

1. Prepare the inhibitor stock at 10 mM concentration in DMSO. Make 16 different concentrations by 2-fold serial dilutions of the 200 µM marimastat solution in DMSO, mix well after each dilution. The last sample should contain only DMSO (no inhibitor). Dilute the prepared samples 50-fold with the assay buffer, the final DMSO concentration would be 2 %v/v. Mix them well by pipetting.
2. Prepare 1 mM substrate stock solution in DMSO. Dilute 1 mM substrate stock 1:125 with assay buffer yielding 8 µM concentration of substrate.
3. Prepare 8 nM concentration of MMP2 in assay buffer from the 8.4 µM enzyme stock solution (1050-fold dilution of the enzyme).
4. Add 50 µL of 8 nM MMP2 solution to each of 16 wells (final MMP2 concentration 2 nM).
5. Add 50 µL of each inhibitor working solution to the designated wells (final inhibitor concentrations from 1 µM to 0.24 nM). Mix by shaking plate gently.
6. Note: conditions with no added substrate and no added enzyme can serve as necessary negative controls. The positive control could be used a chelating agent EDTA (final 10 mM concentration).

7. Incubate at room temperature for 30 min.
8. Add 100 µL of 8 µM substrate to each well (final substrate concentration 4 µM). Mix by shaking plate gently.
9. Monitor the hydrolysis of substrate at 37 ºC temperature every 2.5 min for 60 min, by measuring the increase in fluorescence with a plate reader at an excitation wavelength of 325 nm and an emission wavelength of 393 nm.
10. Analyze the data to evaluate the initial rate of substrate hydrolysis. Calculate the percent inhibition of MMP2 for each concentration of inhibitor to determine IC_{50} values.