Proposal of Time-dependent Inhibition Study

- Example -

If the compound has not been tested with the target kinase by RBC, the IC_{50} determination is required prior to the proposal.

Objectives:

The customer is interested in following job to determine On-rate of the time-dependent inhibitor which is an irreversible and ATP competitive.

1. One compound for targeting BTK

   Determine K_{obs} values at varied ATP concentration but at fixed substrate concentration.

Materials and reagents:

Compounds: Customer's compound(s)

Kinase reaction buffer: 20 mM HEPES-HCl, pH 7.5, 10 mM $MgCl_2$, 1 mM EGTA, 0.02% Brij35, 0.1 mM Na_3VO_4, 0.02 mg/ml BSA, 2 mM DTT, and 1% DMSO.

Enzyme:
BTK: Recombinant human full-lengthprotein, C-term His-tagged, expressed in insect cells. Mw = 81.3 kDa.

Substrate: pEY

Reaction conditions:
8 nM BTK, 0.2 mg/ml pEY, and varied ATP (see below)

Experimental Procedures:

The kinase assays will be performed at room temperature. The compound with serial dilution in DMSO will be added 10-dose IC_{50} mode into Enzyme/substrate mixture using acoustic technology, and 5 concentrations of ATP will be added immediately following compound addition without pre-incubation to initiate the reaction. The activity will be monitored every 5-15 min for time course. ATP and compound concentrations tested will be as follows:

1. K_{obs} determination for BTK

ATP concentrations tested: 10, 30, 75, 150, and 300 μM ATP

Compound concentrations tested: 10-dose IC_{50} with 3-fold serial dilution started at ? μM.

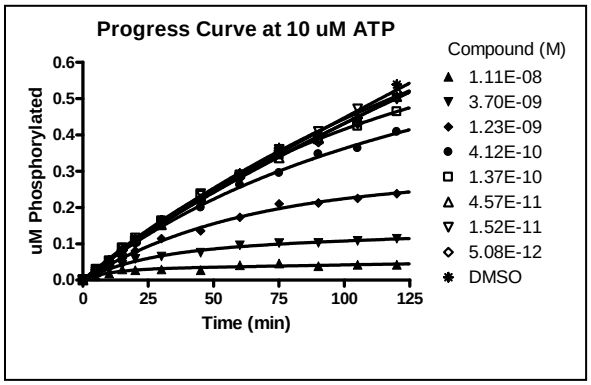
Time points measured: 0, 5, 10, 15, 20, 30, 45, 60, 75, 90, 105, and 120 min

Data Analysis:

If the compound binds to the enzyme time-dependently, the progress curves will show downward curves in dose-dependent manner. The progress curves will be analyzed using equation 1 to obtain K_{obs}.

$$[P] = v_s t + \frac{v_i - v_s}{k_{obs}}[1 - \exp(-k_{obs} t)] \qquad (1)$$

Typical progress curves for time-dependent inhibitor are shown Fig. 1 as an example. If the compound is ATP competitive, the K_{obs} values will be changed by ATP concentrations.



Limitations of this proposal:

1. The time frame of this proposal is 2 hours. If no curving was observed within 2 hours, the above analysis cannot be performed. It will need different experimental designs.
2. If enzyme is not stable for 2 hours and no inhibitor control is curving, the analysis will be performed within the time range which is linear in control.