Biochemical ATP-Competition Assay Service

REACTION BIOLOGY

TARGET-SPECIFIC ASSAY SERVICES

➢ The Service in Brief

The biochemical ATP-competition assay service includes the biochemical determination of the effect of different ATP concentration on the IC_{50} of a compound against a given kinase. The resulting data give information about a possible ATP-competitive mode of action of the test compound, since ATP-competitive compounds show increasing IC_{50} values if tested at increasing ATP-concentrations.

➢ Background

The Ki-values of purely ATP competitive inhibitors can be derived from their IC_{50} values using the Cheng-Prusoff equation (Cheng Y, Prusoff WH (1973) Biochem Pharmacol 22: 3099-3108).
Non-ATP-competitive inhibitors (e.g. allosteric inhibitors) do not show this behaviour, i.e. their IC_{50} values do not change under different ATP-concentrations in a given assay setup. Mixed forms of inhibitors are also possible, which may show an intermediate dependency of the IC_{50} value on the ATP-concentration.

$$K_i = IC_{50} / (1 + \frac{[ATP]}{K_m})$$

Figure 1: Cheng-Prusoff equation
K_i = dissociation constant for binding of the inhibitor to the kinase, "true IC_{50}" ; IC_{50} = measured IC_{50} value for the inhibitor at a distinct assay ATP-concentration: K_m = apparent ATP-K_m of the particular kinase in a given assay setup

➢ Example

IC_{50} values of Staurosporine and the covalent MEK1 inhibitor Selumetinib were determined at different ATP-concentrations. The IC_{50} value of Staurosporine increased 14fold with increasing ATP-concentrations indicating an ATP-competitive mode of action. In contrast increasing ATP-concentration had no effect on the IC_{50} values of Selumetinib indicating that ATP does not compete with this compound.

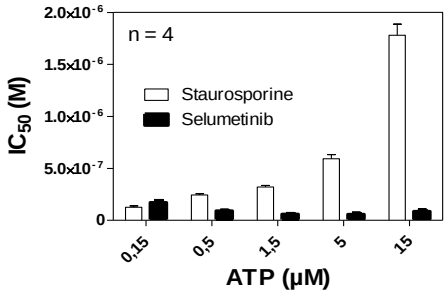

Figure 2: *Dependency of the IC_{50} values of MEK1 inhibitor Selumetinib compared to Staurosporine*

➢ Description of Service

The Service includes three steps:

a) Determination of the linearity of the assay at the ATP-concentration that is 10fold higher than the apparent ATP-K_m of the kinase
b) IC_{50} determination of the compound at the apparent ATP-K_m of the kinase to determine the optimal compound concentration range for IC_{50} measurement.
c) Determination of IC_{50} values at five different ATP-concentrations. Routinely, ATP- concentrations of 0.1/0.3/1/3/10fold of the ATP-Km are used for quadruplicate IC_{50} determination, spanning a maximal ATP-concentration range of factor 100.

All assays are performed as a radiometric, filter-plate based protein kinase activity assay. IC values are determined using 10 different compound concentrations.

Based on the Cheng-Prusoff equation, the IC_{50} values of an ATP-competitive compound would theoretically change under these conditions as follows:

0.1 x ATP-K_m	➡	$IC_{50} = K_i \times 1.1$
0.3 x ATP-K_m	➡	$IC_{50} = K_i \times 1.3$
1 x ATP-K_m	➡	$IC_{50} = K_i \times 2$
3 x ATP-K_m	➡	$IC_{50} = K_i \times 4$
10 x ATP-K_m	➡	$IC_{50} = K_i \times 11$