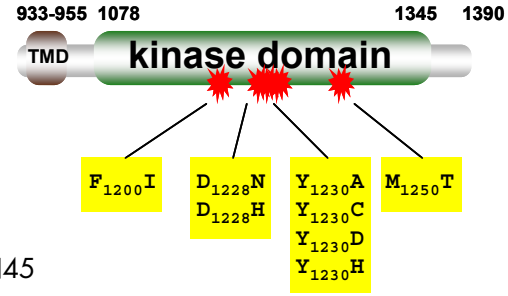
Cellular Phosphorylation Assay for MET Kinase Mutant Panel

REACTION BIOLOGY

CELLULAR ASSAY SERVICES

➢ Phosphorylation Assay Features

- constitutive c-MET phosphorylation induced by designed transmembrane domain
- robust phosphorylation
- standardized assay procedure, HTS feasible
- common Rat1 fibroblast cell background
- real cellular MET Kinase Mutant Panel profiling possible
- reference inhibitors demonstrate comparable inhibition to Reaction Biology standard MET phosphorylation assay using MKN45



➢ Reference Examples

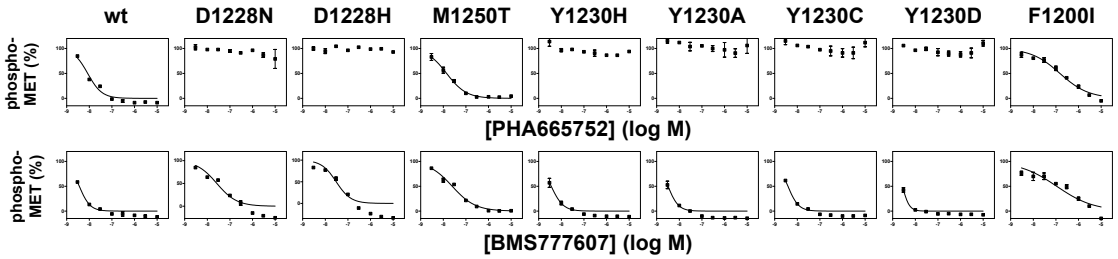
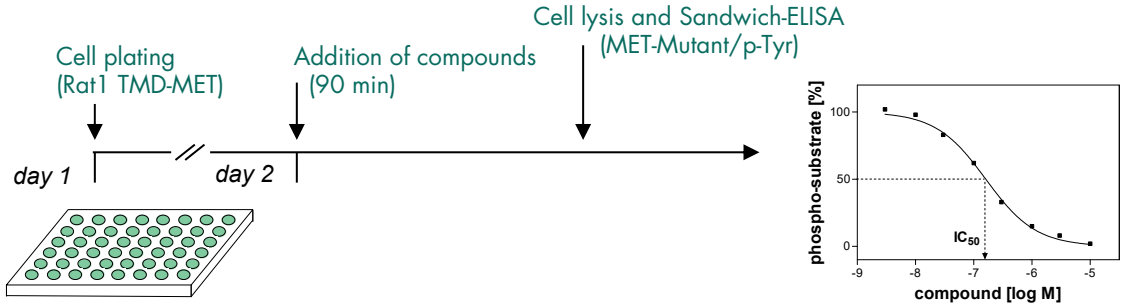


Figure 1: Assay validation.
PHA665752 and BMS777607 were tested for the inhibition of MET phosphorylation on the MET Mutant Panel. PHA665752 is a "type I" MET inhibitor and BMS777607 a "type II" MET inhibitor. "Type I" MET inhibitors have a u-shaped geometry and need tyrosine 1230 (Y1230) for binding, whereas "type II" inhibitors don't need no tyrosine 1230[1]. The graphs show the result of a representative experiment and emphasize the need of tyrosine 1230 for PHA665752 inhibition of MET kinase. The MET Mutations (e.g. D1228N) and wildtype form (wt) are depicted.

[1] Undiner et al. (2010). Anti-Cancer Agents in Medicinal Chemistry **10:** 7-27

➢ You ship your compounds – Reaction Biology performs the testing



- IC_{50} values are determined by testing 8 compound concentrations in semi-logarithmic steps (each concentration in duplicates).
- Quality assurance is provided by calculation of Z' factors for Low/High controls on each assay plate and by including a full IC_{50} curve for a reference inhibitor to monitor adequate dose/response relation in your assay run.

➢ Related Products

- Recombinant MET Kinase Mutant Panel
- Biochemical MET Kinase Mutant Panel screening
- Cellular Soft Agar Growth with MET Kinase Mutants