

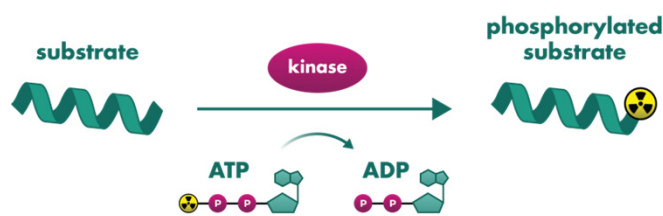
➤ In Brief

Reaction Biology offers a variety of kinase assay panels to determine the selectivity of new kinase inhibitors including broad kinome panels as well as smaller panels focussing on a kinase family such as CDKs or mutants such as the EGFR mutant panel.

➤ Screening formats and research locations

Reaction Biology performs kinase screening at two research locations, Malvern, PA, USA and Freiburg, Germany.

Protein kinases are screened at both facilities with radiometric kinase activity assays. The readout is based on the transfer of ^{33}P -labelled phosphate from ATP to the kinase substrate. The methods, however, differ with respect to retention and detection of the phosphorylated substrates.



Proteins and peptides in the HotSpot™ assay (performed in the US) are captured via spotting of the reaction mix on a filter membrane. The ^{33}P anQinase™ assay (performed in Germany) is performed with scintillator-coated microtiter plates.

Lipid kinases are screened with the kinase activity assay format ADP-Glo at both facilities.

➤ Broad Kinome Selectivity Panels

Panels run at US facility	Panel size	Panel
Wild Type Kinase Panel	380	➤ view panel
Mutant Kinase Panel	343	➤ view panel
Atypical Kinase Panel	24	➤ view panel
Lipid Kinase Panel	21	➤ view panel
Diacylglycerol Kinase Panel	10	➤ view panel

Panels run at German facility	Panel size	Panel
IC50 Wild Type Kinase Panel	340	➤ view panel
Wild Type Kinase Panel	340	➤ view panel
Mutant Kinase Panel (add on)	96	➤ view panel
Lipid Kinase Panel (add on) – also available as standalone Lipid Kinase Profiler	14	➤ view panel

➤ Screening Setup

US facility:

Screening is available in 2 data point mode (1 concentration in duplicates) or IC50 value mode with 5 or 10 concentrations. ATP concentrations can be chosen to be 1 μM or 10 μM or apparent ATP-K_m up to 100 μM . Runs occur twice per month. Controls: vehicle control and for every kinase, we include a kinase-specific control compound in 10-dose IC50 format.

German facility:

Screening is available in 2 data point mode (1 concentration in duplicate or 2 concentrations in singlicate) or IC50 value mode with 6 concentrations (IC50 Wild Type Kinase Panel) or 10 concentrations (Lipid Kinase Panel). Screening is performed at apparent ATP K_m. Runs occur once per month or upon request. Control: vehicle control.

➤ Choosing the Right Screening Mode

2 point profiling can be applied to:

- selectivity testing to get a first idea of the selectivity profile of a compound
- identification of orphan targets

IC50 based profiling is designed:

- to obtain an accurate guiding parameter for optimization of kinase selectivity during the medicinal chemical optimization phase
- to ensure that observed functional effects of a kinase inhibitor are not due to the inhibition of off-target kinases and to identify potential adverse side effects of the drug
- accurate comparison to reference or competitor compounds

➤ Study Example: Wild Type Kinase Panel in IC50 Screening Mode

IC50 values of kinase inhibitor Sorafenib are based on measurement of 6 concentrations (6 log steps; 0.1 nM to 10,000 nM). A threshold value of 20 fold below the IC50 measured with the most sensitive kinase is indicated by a green line.



➤ Study Example: Wild Type Kinase Panel in 2 Data Point Screening Mode

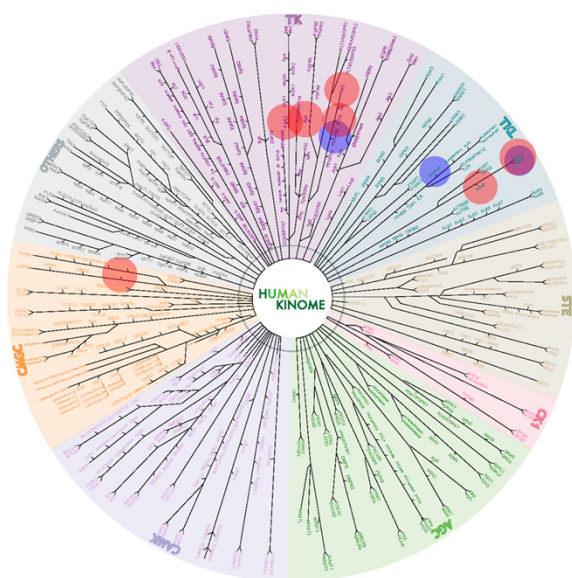
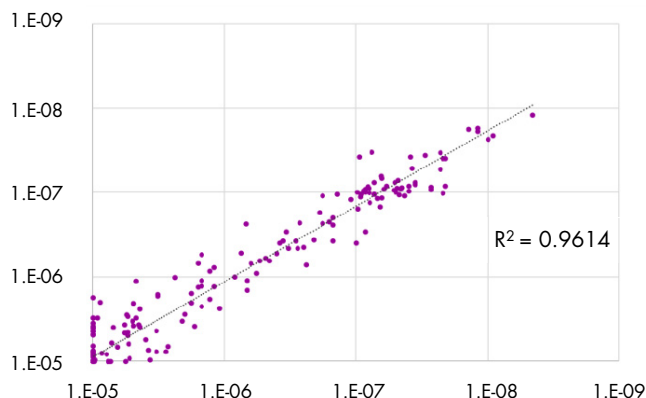
Exemplary results of testing Sorafenib at four different compound concentrations. The green line indicates 50% inhibition.

The figure shows that selection of the most relevant compound concentration is crucial to obtain meaningful results. Sorafenib is best screened at 1000 nM.



➤ Reproducibility

Selectivity profiles of two independent experiments with Ponatinib.



➤ Kinase Mapper Tool

Kinase profiling results can be visualized with our kinase mapper tool (US). For some clients, the visualization is part of the standard report for Wild Type Panel screening (Germany). The Kinase Mapper enables easy comparison of selectivity profiles of compounds.

➤ Custom-tailored Kinase Drug Discovery

Subsequent to broad panel selectivity screening we can validate results with IC50 value determination of individual kinases.

Selective drug candidates that are chosen for further development can be tested with our biochemical, biophysical, and cell-based assay suite to determine:

- Binding affinity
- Binding kinetics
- Stoichiometry
- Thermodynamic parameters
- Substrate competition
- Mode of inhibition
- Intracellular binding affinity and target occupation rate

[Reach out to our client service team today](#) to discuss your research needs.