

➤ Available cellular phosphorylation assays to screen your compounds

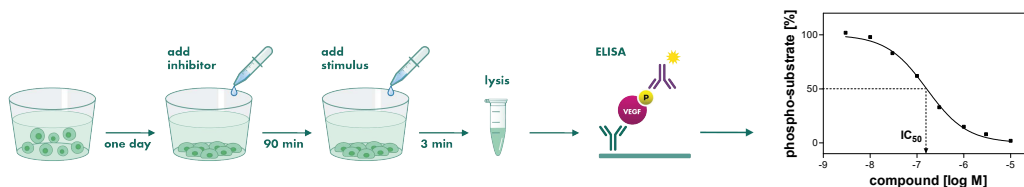
We are kinase experts and believe that especially in the field of rationale drug development of small molecule kinase inhibitors, a compound ranking based on cellular data is pivotal for the decision on further preclinical testing. After *in vitro* kinase profiling of your compounds a similar experimental setting based on cellular phosphorylation assays will substantially increase your knowledge on which one of your inhibitors are still blocking the kinase in the cellular context. In addition, cell-based test systems offer the advantage of physiological ATP concentrations at the site of action and the phosphorylation of physiological substrates.

Generally, cells are preincubated with test compounds to allow thorough target binding. In case of receptor tyrosine kinases we determine the autophosphorylation level, for serine/threonine kinases we have developed assays that allow the quantification of phospho-levels of the immediate downstream substrate. Please check for your kinase of interest.

➤ Cellular phosphorylation assays complement your rational drug development

Kinase	Cell Line	Transfected	Type of Kinase	Origin: Kinase / Cell Line	Quantified phospho-Protein
AKT1	Rat1-myrAKT1	yes	Ser/Thr Kinase	human / rat	GSK3-beta
ALK	Karpas-299	no	RTK	human / human	ALK (autophosphorylation)
Aurora-B	HT-29	no	Ser/Thr Kinase	human / human	Histone H3
AXL	MEF-AXL	yes	RTK	human / mouse	AXL (autophosphorylation)
BCR-ABL	K562	no	RTK	human / human	BCR-ABL (autophosphorylation)
B-RAF-VE	Rat1-B-RAF-VE	yes	Ser/Thr Kinase	human / rat	MEK1
EGF-R	A431	no	RTK	human / human	EGF-R (autophosphorylation)
EGF-R Mutant Panel	Rat 1	yes	RTK	human / rat	EGF-R (autophosphorylation)
EPHB4	MEF-EPHB4	yes	RTK	human / mouse	EPHB4 (autophosphorylation)
ERBB2	NIH3T3-ERBB2	yes	RTK	human / mouse	ERBB2 (autophosphorylation)
ERBB4	T47D	no	RTK	human / human	ERBB4 (autophosphorylation)
FAK	MEF-FAK	yes	Non-receptor TK	human / mouse	FAK (autophosphorylation)
FGF-R2	Kato-III	no	RTK	human / human	FGF-R2 (autophosphorylation)
FLT3-wt	MEF-FLT3-wt	yes	RTK	human / mouse	FLT3-wt (autophosphorylation)
FLT3-DY	MEF-FLT3-DY	yes	RTK	human / mouse	FLT3-DY (autophosphorylation)
FLT3-ITD	MEF-FLT3-ITD	yes	RTK	human / mouse	FLT3-ITD (autophosphorylation)
Haspin	HT-29	no	Ser/Thr Kinase	human / human	Histone H3
IGF1-R	MEF-IGF1-R	yes	RTK	human / mouse	IGF1-R (autophosphorylation)
KIT	M07e	no	RTK	human / human	KIT (autophosphorylation)
MET	MKN45	no	RTK	human / human	MET (autophosphorylation)
MET Mutant Panel	Rat 1	yes	RTK	human / rat	MET (autophosphorylation)
MNK1	Karpas-299	no	Ser/Thr Kinase	human / human	eIF4E (autophosphorylation)
PDGFR-beta	NIH3T3	no	RTK	mouse / mouse	PDGFR-beta (autophosphorylation)
PIM1	HEK293	yes	Ser/Thr Kinase	human / human	Bad
PIM2	HEK293	yes	Ser/Thr Kinase	human / human	Bad
PIM3	HEK293	yes	Ser/Thr Kinase	human / human	Bad
ROCK	A7r5	no	Ser/Thr Kinase	rat / rat	Myosin light chain
RON	T47D	no	RTK	human / human	RON (autophosphorylation)
S6K	Karpas-299	no	Ser/Thr Kinase	human / human	Ribosomal protein S6
SRC	MEF-SRC	yes	Non-receptor TK	human / mouse	SRC (autophosphorylation)
VEGF-R2	HUE	no	RTK	human / human	VEGF-R2 (autophosphorylation)
VEGF-R3	MEF-VEGF-R3	yes	RTK	human / mouse	VEGF-R3 (autophosphorylation)

➤ You ship your compounds – Reaction Biology performs the testing



- IC₅₀ 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 IC₅₀ curve for a reference inhibitor to monitor adequate dose/response relation in your assay run.