Cellular Phosphorylation Assays

REACTION BIOLOGY

CELLULAR ASSAY SERVICES

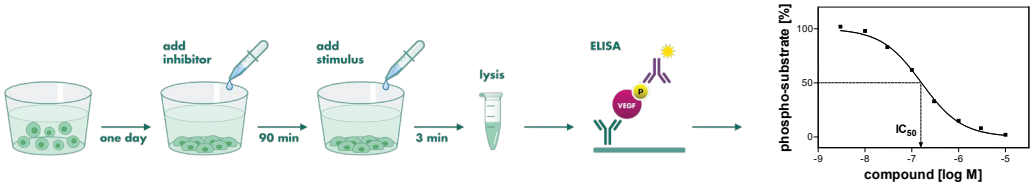
➢ Available cellular phosphorylation assays to screen your compounds

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)
TIE2	CHO-TIE2	yes	RTK	human / hamster	**TIE2** (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



- 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.