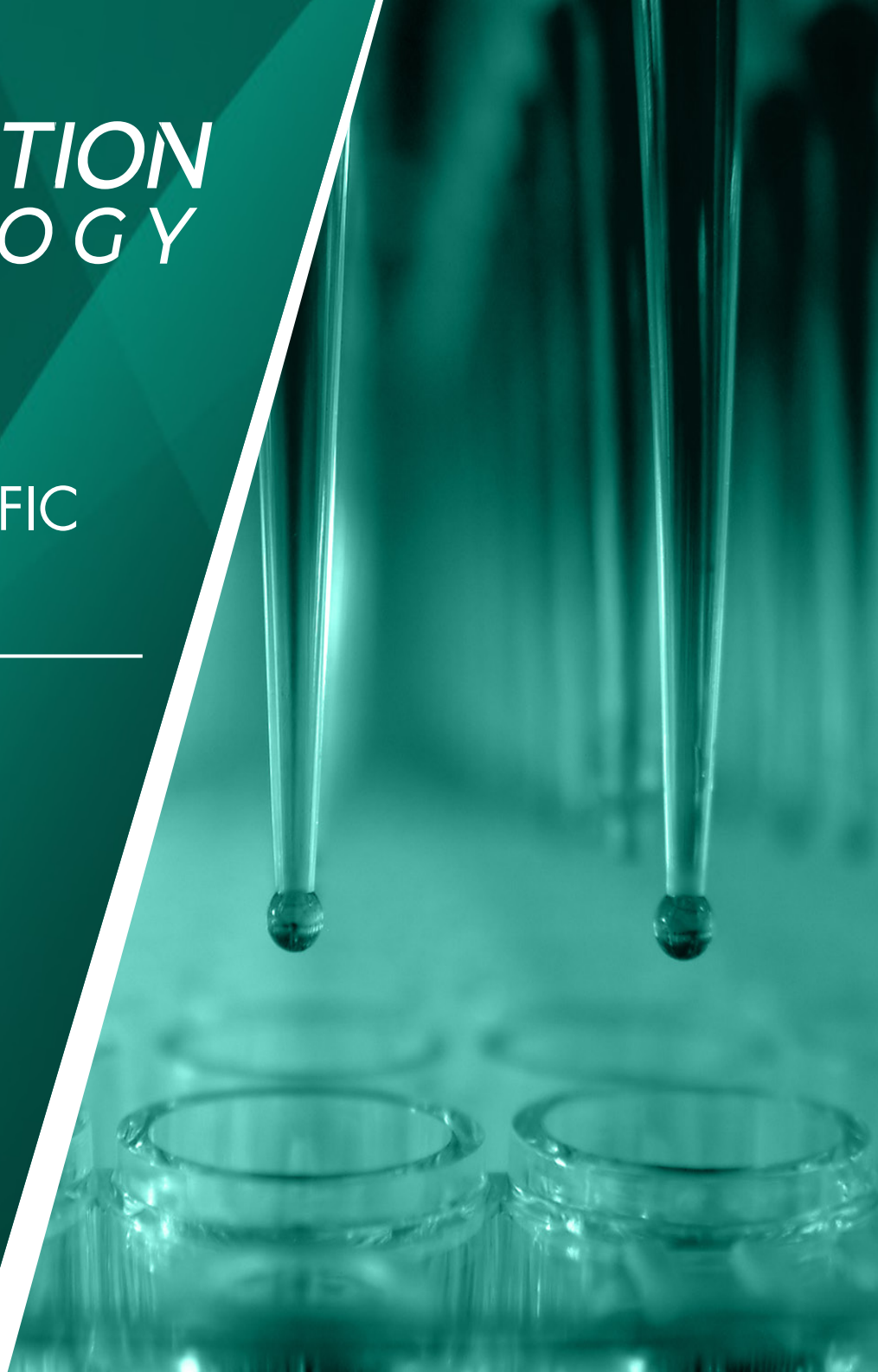
REACTION BIOLOGY

Target-Specific Assays

- Kinase Assays
- Epigenetic Assays
- RAS Assays
- Targeted Protein Degradation Assays
- Protease Assays
- Phosphatase Assays
- Ion Channels
- and more



Reaction Biology has provided their service for numerous projects with us, especially on multiple kinase assays which have guided our way through drug development. I was very impressed by their level of expertise and professionalism. The insightful advice they provide has been instrumental to our successes. The reproducibility, quality, and reporting of the results have been outstanding throughout the many years we have been working together.

Dr Laurent Meijer

Chair & Chief Scientific Officer at
Perha Pharmaceuticals & ManRos Therapeutics

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Target-Specific Assays

Reaction Biology offers a variety of target-specific assays for drug discovery. Most of our assays are high-throughput compatible and can be customized to your specific requirements.

Kinase Assays

Your trusted CRO for kinase drug discovery

Reaction Biology offers the most used and most valued kinase discovery platform in the industry comprising enzymatic, biophysical and cell-based assays as well as in vivo models that target kinase-related diseases.

Throughout the history of our company, our scientists were dedicated to use their expertise to advance kinase inhibitors which are now used in the clinic on a daily basis.

Our clients have identified us as their favorite CRO

HTStec Kinase Profiling Trends Survey has reported that the Reaction Biology kinase screenning is the most used platform in industry. Almost half of those interviewed named Reaction Biology their favourite CRO.

Quality of assays

Reaction Biology uses state-of-the-art radioactivity-based assays for enzymatic kinase inhibitor screening. The assay directly measures the activity of the kinase with high reproducibility and avoids false negative and positives common with other kinase assay formats.

Customer service

We understand the needs of small and large research organizations alike. Our scientists are dedicated to providing immediate technical support and advice for every research project we undertake. Call us anytime.

Flexibility

We provide fee-for-service, customized projects as well as integrated drug discovery services. We can custom-tailor existing assays and develop new assays making us the CRO of choice for every project.

Expertise

Reaction Biology has been serving clients for over 20 years. Our staff includes expert scientists from a variety of research backgrounds who are available to assist you at each phase of the drug discovery process: hit identification, hit to lead and lead optimization.

Kinase assays at Reaction Biology screening facilities

With the largest portfolio of kinase assays available for drug discovery and 20 years of expertise in custom-tailored assay development, Reaction Biology is guaranteed to provide the kinase assay you need.

Explore our portfolio of 860+ kinase assays offered with a variety of assay formats:
Biochemical assays:

- Radiometric activity assays (HotSpot performed in US, ^{33}PanQinase performed in Germany)
- Luminescence-based activity assays (ADP-Glo performed in US and Germany)
- HTRF-based activity assay (performed in US)

Cell-based assays:

- Kinase activity testing via Cell Phosphorylation assay format
- Target engagement via NanoBRET assay format

Kinase	Assay Format
AAK1	NB
ABL1	HS, PQ, NB
ABL1 (E255K)	HS, PQ, NB
ABL1 (E255V)	HS
ABL1 (F317I)	HS, PQ, NB
ABL1 (F317L)	HS, NB
ABL1 (G250E)	HS, PQ
ABL1 (H396P)	HS, PQ, NB
ABL1 (M351T)	HS, PQ, NB
ABL1 (Q252H)	HS, PQ, NB
ABL1 (T315I)	HS, PQ, NB
ABL1 (V299L)	HS
ABL1 (Y253F)	HS, PQ, NB
ABL1 (Y253H)	HS
ABL2	HS, PQ, NB
ACVR1	HS, PQ, NB
ACVR1 (G328V)	NB
ACVR1 (G356D)	NB
ACVR1 (Q207D)	HS, NB
ACVR1 (R206H)	HS, NB
ACVR1B	HS, PQ, NB

Kinase	Assay Format
ACVR2A	PQ
ACVR2B	PQ
ACVRL1	HS, PQ, NB
ADK	NB
AKT1	HS, PQ, NB, CPA
AKT1 (aa106-480)	PQ
AKT1 (E17K)	HS, NB
AKT2	HS, PQ, NB
AKT2 (aa107-481)	PQ
AKT2 (E17K)	HS, NB
AKT3	HS, PQ
AKT3 (aa106-479)	PQ
AKT3 (E17K)	HS, NB
AKT3 (G171R)	HS, NB
ALK	HS, PQ, CPA
ALK (C1156Y)	HS, PQ
ALK (F1174L)	HS, PQ
ALK (F1174L)-EML4	HS, PQ
ALK (F1174L)-NPM1	HS, PQ
ALK (F1174S)	HS, PQ
ALK (G1202R)	HS, PQ

Kinase	Assay Format
ALK (G1269A)	HS
ALK (G1269S)	HS
ALK (L1152R)	HS
ALK (L1196M)	HS, PQ
ALK (L1196M/G1202R)	HS
ALK (R1275Q)	HS, PQ
ALK (S1206R)	HS
ALK (T1151-L1152insT)	HS
ALK (T1151M)	HS
ALK-EML4	HS, PQ
ALK-KIF5B	HS
ALK-KLC1	HS
ALK-NPM1	HS, PQ
ALK-STRN	HS
ALK-TFG	HS
ALK-TPM1	HS
ALK-TPM3	HS
ARAF (Y301D/Y302D)	HS, PQ
ATM	HS
AURKA	HS, PQ, NB
AURKB	HS, PQ, NB, CPA

Kinase	Assay Format
AURKB (G160L)	HS
AURKC	HS, PQ, NB
AXL	HS, PQ, NB, CPA
AXL (R199C)	HS
BCR-ABL1	CPA
BLK	HS, PQ, NB
BMP2K	NB
BMPR1A	HS, PQ, NB
BMPR1B	HS, PQ
BMPR2	HS
BMX	HS, PQ, NB
BRAF	HS, PQ
BRAF (d485-489/P490Y)	HS
BRAF (G464V)	HS
BRAF (G469A)	HS
BRAF (K601E)	HS
BRAF (L597V)	HS
BRAF (R506_K507insVLR)	HS
BRAF (T599_V600insT)	HS
BRAF (V600A)	HS
BRAF (V600D)	HS
BRAF (V600E)	HS, PQ, NB, CPA
BRAF (V600K)	HS
BRAF-FAM131B	HS
BRAF-KIAA1549 (Kex15Bex9)	HS
BRAF-KIAA1549 (Kex16Bex9)	HS
BRAF-SRGAP3	HS
BRSK1	HS, PQ, NB
BRSK2	HS, PQ, NB
BTK	HS, PQ, NB
BTK (C481S)	HS, NB
BTK (E41K)	HS, NB
BTK (P190K)	HS, NB
BTK (T474I)	HS
BTK (Y485F)	HS
BUB1B	PQ
CAMK1	HS, NB
CAMK1D	HS, PQ, NB
CAMK1G	HS, NB

Kinase	Assay Format
CAMK2A	HS, PQ, NB
CAMK2B	HS, PQ
CAMK2D	HS, PQ, NB
CAMK2G	HS, PQ, NB
CAMK4	HS, PQ
CAMKK1	HS, PQ
CAMKK2	HS, PQ
CDC42BPA	HS, PQ
CDC42BPB	HS, PQ
CDC42BPG	HS
CDC7-DBF4	HS, PQ
CDK1-CCNA2	HS, PQ
CDK1-CCNB1	HS, PQ, NB
CDK1-CCNE1	HS, PQ, NB
CDK2-CCNA1	HS, NB
CDK2-CCNA2	HS, PQ
CDK2-CCND1	PQ
CDK2-CCNE1	HS, PQ, NB
CDK2-CCNE2	HS
CDK2-CCNO	HS
CDK3-CCNC	HS, PQ
CDK3-CCNE1	HS, PQ, NB
CDK3-CCNE2	HS
CDK4-CCND1	HS, PQ, NB
CDK4-CCND2	HS, PQ
CDK4-CCND3	HS, PQ, NB
CDK5-CDK5R1	HS, PQ, NB
CDK5-CDK5R1 (p25)	HS, PQ
CDK5-CDK5R2	NB
CDK6-CCND1	HS, PQ, NB
CDK6-CCND2	HS, PQ
CDK6-CCND3	HS, PQ, NB
CDK7	NB
CDK7-CCNH	NB
CDK7-CCNH-MNAT1	HS, PQ
CDK8-CCNC	HS, PQ, NB
CDK9-CCNK	HS, PQ, NB
CDK9-CCNT1	HS, PQ, NB
CDK9-CCNT2	HS, NB

Kinase	Assay Format
CDK10-CCNL2	NB
CDK10-CCNQ	PQ
CDK11A-CCNK	NB
CDK11A-CCNL2	NB
CDK11B-CCNK	PQ
CDK12 (R722C)-CCNK	HS, PQ
CDK12-CCNK	HS, PQ, NB
CDK13-CCNK	HS, PQ, NB
CDK14-CCNY	HS, PQ, NB
CDK15-CCNA2	HS, PQ
CDK15-CCNB1	HS, PQ
CDK15-CCNY	NB
CDK16-CCNY	HS, PQ, NB
CDK17-CCNY	HS, NB
CDK17-CDK5R1	PQ
CDK18-CCNY	HS, PQ, NB
CDK19-CCNC	HS, PQ, NB
CDK20-CCNH	PQ, NB
CDK20-CCNT1	PQ
CDKL1	NB
CDKL2	NB
CDKL3	NB
CDKL5	NB
CEP43-FGFR1	HS
CHEK1	HS, PQ, NB
CHEK2	HS, PQ, NB
CHEK2 (I157T)	HS
CHUK	HS, PQ
CILK1	HS, NB
CIT	HS
CK1a1 (E42C)	HS
CK1a1 (I35C)	HS
CLK1	HS, PQ, NB
CLK2	HS, PQ, NB
CLK3	HS, PQ
CLK4	HS, PQ, NB
COQ8B	NB
CSF1R	HS, PQ, NB
CSK	HS, PQ, NB

Kinase	Assay Format
CSNK1A1	HS, PQ
CSNK1A1L	HS, NB
CSNK1D	HS, PQ, NB
CSNK1E	HS, PQ, NB
CSNK1E (R178C)	HS
CSNK1G1	HS, PQ
CSNK1G2	HS, PQ, NB
CSNK1G3	HS, PQ
CSNK2A1	HS, PQ, NB
CSNK2A2	HS, PQ, NB
DAPK1	HS, PQ
DAPK2	HS, PQ, NB
DAPK3	HS, PQ
DCLK1	HS
DCLK2	HS, PQ
DCLK3	NB
DDR1	HS, NB
DDR2	HS, PQ, NB
DDR2 (N456S)	HS, PQ, NB
DDR2 (T654M)	HS, PQ
DGKA	ADP-Glo (US)
DGKB	ADP-Glo (US)
DGKD	ADP-Glo (US)
DGKE	ADP-Glo (US)
DGKG	ADP-Glo (US)
DGKH	ADP-Glo (US)
DGKI	ADP-Glo (US)
DGKK	ADP-Glo (US)
DGKQ	ADP-Glo (US)
DGKZ	ADP-Glo (US)
DMPK	HS, PQ, NB
DSTYK	HS, PQ
DYRK1A	HS, PQ, NB
DYRK1B	HS, PQ, NB
DYRK2	HS, PQ, NB
DYRK3	HS, PQ
DYRK4	HS, PQ
EEF2K	HS, PQ
EGFR	HS, PQ, CPA, BaF3

Kinase	Assay Format
EGFR (A763_Y764insFHEA)	HS
EGFR (A763_Y764insFQEA)	HS
EGFR (A767_S768insTLA)	HS
EGFR (C775S/T790M/L858R)	HS
EGFR (C797A)	HS
EGFR (C797G/L858R)	HS
EGFR (C797S)	HS, PQ
EGFR (C797S/L858R)	HS, PQ
EGFR (d746)	HS
EGFR (d746-750)	HS, PQ
EGFR (d746-750/C775S/T790M/L858R)	HS, PQ
EGFR (d746-750/C797A)	HS
EGFR (d746-750/C797S)	HS, PQ, CPA
EGFR (d746-750/G724S)	HS
EGFR (d746-750/S768I)	HS
EGFR (d746-750/T790M)	HS, CPA
EGFR (d746-750/T790M/C797S)	HS, PQ, CPA, BaF3
EGFR (d746-750/T790M/C797S/L858R)	HS
EGFR (d746-750/T790M/L792F)	HS
EGFR (d746-750/T790M/L792H)	HS
EGFR (d746-750/T790M/L798I)	HS
EGFR (d746-750/T790M/L858R)	HS
EGFR (d747-749)	HS
EGFR (d747-749/A750P)	HS, PQ, CPA
EGFR (d747-752/P753S)	HS, PQ
EGFR (d752-759)	HS, PQ, CPA
EGFR (D761Y)	HS
EGFR (D770_N771insNPG)	HS
EGFR (D770_N771insNPG/T790M)	HS
EGFR (D770GY)	HS
EGFR (G719C)	HS, PQ
EGFR (G719D)	HS
EGFR (G719S)	HS, PQ, CPA
EGFR (K716A)	HS

Kinase	Assay Format
EGFR (K716A/C797S/L858R)	HS
EGFR (K716A/T790M/C797S/L858R)	HS
EGFR (K716Q/L718Q)	HS
EGFR (K728A)	HS
EGFR (K728A/T790M/C797S/L858R)	HS
EGFR (L718Q)	HS, PQ
EGFR (L747S)	HS
EGFR (L792F)	HS
EGFR (L792F/L858R)	HS
EGFR (L792H)	HS
EGFR (L792H/C797S/L858R)	HS
EGFR (L792H/L858R)	HS
EGFR (L858R)	HS, PQ, CPA
EGFR (L861Q)	HS, PQ, CPA
EGFR (N771_P772insH)	HS
EGFR (R999A)	HS
EGFR (T790M)	HS, PQ, CPA
EGFR (T790M/C797G/L858R)	HS
EGFR (T790M/C797S)	HS
EGFR (T790M/C797S/L858R)	HS, PQ, CPA
EGFR (T790M/L792F/C797S/L858R)	HS
EGFR (T790M/L792F/L858R)	HS
EGFR (T790M/L792H/C797S/L858R)	HS
EGFR (T790M/L792H/L858R)	HS
EGFR (T790M/L858R)	HS, PQ, CPA
EGFR (V769_D770insGE)	HS
EIF2AK1	HS, PQ
EIF2AK2	HS, PQ
EIF2AK3	HS, PQ
EIF2AK4	HS, PQ, NB
EPHA1	HS, PQ, NB
EPHA2	HS, PQ, NB
EPHA3	HS, PQ, NB
EPHA4	HS, PQ, NB
EPHA5	HS, PQ, NB
EPHA6	HS, PQ, NB

Kinase	Assay Format
EPHA7	HS, PQ, NB
EPHA8	HS, PQ, NB
EPHB1	HS, PQ, NB
EPHB2	HS, PQ, NB
EPHB3	HS, PQ, NB
EPHB4	HS, PQ, NB, CPA
ERBB2	HS, PQ, CPA
ERBB2 (775YVMA776)	PQ
ERBB2 (A775_G776insYVMA)	HS
ERBB2 (D769H)	HS
ERBB2 (D769Y)	HS
ERBB2 (P1170A)	HS
ERBB2 (P780-Y781insGSP)	HS
ERBB2 (R896C)	HS
ERBB2 (V777_G778insCG)	HS
ERBB2 (V777L)	HS
ERBB4	HS, PQ, CPA
ERN1	HS, NB
ERN1 (R727A)	HS
ERN1 (R728A)	HS
ERN1/IRE1 (R727A/R728A)	HS
ERN2	HS, NB
FER	HS, PQ, NB
FES	HS, PQ, NB
FGFR1	HS, PQ, NB
FGFR1 (V561M)	HS, PQ
FGFR1 (W666R)	HS
FGFR2	HS, PQ, NB, CPA
FGFR2 (C491A)	HS
FGFR2 (C491F)	HS
FGFR2 (C491S)	HS
FGFR2 (E565G)	HS
FGFR2 (K526E)	HS
FGFR2 (K641R)	HS
FGFR2 (K659N)	HS
FGFR2 (N549H)	HS
FGFR2 (R612T)	HS
FGFR2 (V564F)	HS
FGFR2 (V564L)	HS

Kinase	Assay Format
FGFR3	HS, PQ, NB
FGFR3 (G697C)	HS, PQ, NB
FGFR3 (K650E)	HS, PQ
FGFR3 (K650M)	HS, PQ
FGFR3 (K650Q)	HS
FGFR3 (V555M)	HS
FGFR4	HS, PQ, NB
FGFR4 (N535K)	HS, PQ
FGFR4 (V550E)	HS, PQ
FGFR4 (V550L)	HS
FGFR4 (V550M)	HS
FGR	HS, PQ, NB
FLT1	HS, PQ, NB
FLT3	HS, PQ, NB, CPA
FLT3 (D835H)	NB
FLT3 (D835V)	NB
FLT3 (D835Y)	HS, PQ, NB, CPA
FLT3 (F594_R595insR)	HS
FLT3 (F594_R595insREY)	HS
FLT3 (ITD)	HS, PQ, CPA
FLT3 (ITD)-NPOS	HS
FLT3 (ITD)-W51	HS
FLT3 (K663Q)	NB
FLT3 (N841I)	NB
FLT3 (R595_E596insEY)	HS
FLT3 (R834Q)	NB
FLT3 (Y591_V592insVDFREYEYD)	HS
FLT4	HS, PQ, CPA
FRK	HS, PQ, NB
FYN	HS, PQ, NB, CPA
FYN (Y531F)	HS, PQ, NB
GAK	NB
GRK1	HS
GRK2	HS, PQ
GRK3	HS, PQ
GRK4	HS, PQ
GRK5	HS, PQ
GRK6	HS, PQ

Kinase	Assay Format
GRK7	HS, PQ
GSG2	HS, PQ, CPA
GSK3A	HS, PQ, NB
GSK3B	HS, PQ, NB
HCK	HS, PQ, NB
HIPK1	HS, PQ
HIPK2	HS, PQ, NB
HIPK3	HS, PQ, NB
HIPK4	HS, PQ, NB
HUNK	NB
IGF1R	HS, PQ, NB, CPA
IKBKB	HS, PQ
IKBKE	HS, PQ, NB
INSR	HS, PQ, NB
INSRR	HS, PQ
IRAK1	HS, PQ, NB
IRAK2	HS
IRAK3	NB
IRAK4	HS, PQ, NB
IRAK4 (aa104-460) untagged	PQ
ITK	HS, PQ, NB
JAK1	PQ
JAK1 (aa850-1154)	PQ
JAK1 (aa866-1154)	HS
JAK1 (S729C)	PQ
JAK2	HS, PQ, NB
JAK2 (JH1&2)	HS
JAK2 (JH1)	NB
JAK2 (V617F)	HS, NB
JAK3	HS, PQ, NB
KDR	HS, PQ, CPA
KIT	HS, PQ, NB, CPA
KIT (A829P)	HS, PQ, NB
KIT (d557-558)	HS
KIT (D816E)	HS, PQ
KIT (D816F)	HS
KIT (D816H)	HS, NB
KIT (D816I)	HS
KIT (D816V)	HS, PQ, NB

Kinase	Assay Format
KIT (D816Y)	HS
KIT (D820E)	HS
KIT (D820Y)	HS
KIT (K642E)	HS
KIT (L576P)	NB
KIT (T670I)	HS, PQ
KIT (V559A)	HS
KIT (V559D)	HS, PQ, NB
KIT (V559D/T670I)	HS, PQ, NB
KIT (V559D/V654A)	HS, PQ, NB
KIT (V560G)	HS, PQ
KIT (V560G/D816V)	HS
KIT (V560G/N822K)	HS
KIT (V654A)	HS, PQ
KIT (Y823D)	HS
KSR1	HS
KSR1 (A635F)	HS
KSR1 (L639F)	HS
KSR2	HS
KSR2 (R676S)	HS
LATS1	HS, NB
LATS2	HS, NB
LCK	HS, PQ, NB
LIMK1	HS, PQ, NB
LIMK2	HS, PQ, NB
LRRK2	HS, PQ, NB, CPA
LRRK2 (G2019S)	HS, PQ, NB
LRRK2 (I2020T)	HS, PQ, NB
LRRK2 (R1441C)	HS, PQ, NB
LTK	HS, PQ, NB
LYN	HS, PQ, NB
LYN (d23-43)	HS
MAK	HS
MAP2K1	HS, PQ
MAP2K1 (F53L)	PQ
MAP2K1 (P124L)	HS, PQ
MAP2K1 (S218E/S222E)	PQ
MAP2K1/KRAS(G12C)	NB
MAP2K1/MAP2K2	CPA

Kinase	Assay Format
MAP2K2	HS, PQ
MAP2K2/KRAS(G12C)	NB
MAP2K3	HS
MAP2K4	HS, PQ
MAP2K5	HS, PQ
MAP2K6	HS, NB
MAP2K6 (S207D/T211D)	PQ
MAP2K7	HS, PQ
MAP3K1	HS, PQ
MAP3K2	HS, PQ, NB
MAP3K3	HS, PQ, NB
MAP3K4	NB
MAP3K5	HS, PQ
MAP3K6	HS
MAP3K7	HS
MAP3K7-TAB1	PQ
MAP3K8	HS, PQ
MAP3K9	HS, PQ, NB
MAP3K10	HS, PQ, NB
MAP3K11	HS, PQ, NB
MAP3K12	HS, NB
MAP3K13	NB
MAP3K14	HS, PQ
MAP3K19	HS, NB
MAP3K20	HS, PQ, NB
MAP3K21	HS, PQ, NB
MAP4K1	HS, NB
MAP4K2	HS, PQ, NB
MAP4K3	HS, NB
MAP4K4	HS, PQ
MAP4K5	HS, PQ, NB
MAPK1	HS, PQ, NB
MAPK3	HS, PQ, NB
MAPK4	NB
MAPK6	NB
MAPK7	HS
MAPK7 (aa5-397)	PQ
MAPK7 (CD)	HS
MAPK8	HS, PQ, NB

Kinase	Assay Format
MAPK9	HS, PQ, NB
MAPK10	HS, PQ, NB
MAPK11	HS, PQ, NB
MAPK12	HS, PQ
MAPK13	HS, PQ
MAPK14	HS, PQ, NB
MAPK14 (T106M)	HS, NB
MAPK15	HS, PQ
MAPKAPK2	HS, PQ
MAPKAPK3	HS, PQ
MAPKAPK5	HS, PQ
MARK1	HS, PQ
MARK2	HS, PQ, NB
MARK3	HS, PQ, NB
MARK4	HS, PQ, NB
MAST3	HS, NB
MAST4	NB
MASTL	HS, PQ
MATK	HS, PQ
MELK	HS, PQ, NB
MELK (T460M)	HS, NB
MERTK	HS, PQ, NB
MERTK (A708S)	HS, NB
MET	HS, PQ, NB, CPA
MET (D1228A)	HS
MET (D1228G)	HS
MET (D1228H)	HS, PQ, NB
MET (D1228N)	HS, PQ, NB, CPA
MET (D1228V)	HS
MET (D1228Y)	HS
MET (D1288H)	CPA
MET (DelEx14)	HS
MET (F1200I)	HS, PQ, NB, CPA
MET (G1163R)	HS, PQ
MET (H1094L)	HS
MET (H1094Y)	HS
MET (K1244R)	HS
MET (L1195F)	HS
MET (L1195V)	HS, PQ

Kinase	Assay Format
MET (M1250I)	HS
MET (M1250T)	HS, PQ, NB, CPA
MET (P991S)	HS, NB
MET (R970C)	HS
MET (T1173I)	HS, NB
MET (T992I)	HS, NB
MET (V1092I)	HS, NB
MET (Y1230A)	HS, PQ, NB, CPA
MET (Y1230C)	HS, PQ, NB, CPA
MET (Y1230D)	HS, PQ, NB, CPA
MET (Y1230H)	HS, PQ, NB, CPA
MET (Y1230S)	HS
MET (Y1235D)	HS, PQ, NB
MET-KIF5B	HS
MET-TFG	HS
MINK1	HS, PQ
MKNK1	HS, PQ, CPA
MKNK2	HS, PQ, NB
MOK	NB
MST1R	HS, PQ, NB, CPA
MTOR	HS, PQ
MUSK	HS, PQ, NB
MYLK	HS, PQ
MYLK2	HS, PQ, NB
MYLK3	HS, PQ, NB
MYLK4	HS, NB
MYO3A	HS
MYO3B	HS
NEK1	HS, PQ, NB
NEK2	HS, PQ, NB
NEK3	HS, PQ, NB
NEK4	HS, PQ, NB
NEK5	HS, NB
NEK6	HS, PQ, NB
NEK7	HS, PQ
NEK8	HS
NEK9	HS, PQ, NB
NEK11	HS, PQ, NB
NIM1K	HS, NB

Kinase	Assay Format
NLK	HS, PQ, NB
NRK	NB
NTRK1	HS, PQ, NB
NTRK1 (A608D)	HS
NTRK1 (F589L)	HS
NTRK1 (G595R)	HS
NTRK1 (G595R/A608D)	HS
NTRK1 (G595R/G667A)	HS
NTRK1 (G595R/G667C)	HS
NTRK1 (G595R/G667S)	HS
NTRK1 (G595R/L657M)	HS
NTRK1 (G667C)	HS, PQ, NB
NTRK1 (G667S)	HS
NTRK1 (L657M)	HS
NTRK1-TFG	HS
NTRK1-TPM3	HS
NTRK1-TPR	HS
NTRK2	HS, PQ, NB
NTRK3	HS, PQ
NTRK3 (G623E)	HS
NTRK3 (G623R)	HS
NTRK3 (G623R/L686M)	HS
NTRK3 (G696A)	HS
NTRK3 (L686M)	HS
NUAK1	HS, PQ, NB
NUAK2	HS, PQ, NB
OXSR1	HS
PAK1	HS, PQ
PAK2	HS, PQ
PAK2 (Y443N)	HS
PAK3	HS, PQ
PAK4	HS, PQ, NB
PAK5	HS, PQ, NB
PAK6	HS, PQ, NB
PASK	HS, PQ
PBK	HS, PQ
PDGFRA	HS, PQ
PDGFRA (D842V)	HS, PQ
PDGFRA (G680R)	HS

Kinase	Assay Format
PDGFRA (T674I)	HS, PQ
PDGFRA (V561D)	HS, PQ, NB
PDGFRA-FIP1L1	HS
PDGFRB	HS, PQ, CPA
PDGFRB-TPM3	HS
PDK1	HS, PQ
PDK2	HS
PDK3	HS
PDK4	HS
PDPK1	HS
PEAK1	HS
PHKG1	HS, PQ, NB
PHKG2	HS, PQ, NB
PI4K2A	ADP-Glo (US, Ger)
PI4K2B	ADP-Glo (Ger)
PI4KA	ADP-Glo (US)
PI4KB	ADP-Glo (US, Ger)
PIK3C2A	ADP-Glo (US, Ger)
PIK3C2B	ADP-Glo (Ger)
PIK3C2G	ADP-Glo (Ger)
PIK3C3	ADP-Glo (US, Ger), NB
PIK3CA (C420R)-PIK3R1	NB
PIK3CA (E542K)-PIK3R1	ADP-Glo (US), NB
PIK3CA (E545A)-PIK3R1	NB
PIK3CA (E545K)-PIK3R1	ADP-Glo (US), NB
PIK3CA (H1047L)-PIK3R1	NB
PIK3CA (H1047R)-PIK3R1	ADP-Glo (US), NB
PIK3CA (H1047Y)-PIK3R1	NB
PIK3CA (I800L)-PIK3R1	NB
PIK3CA (M1043I)-PIK3R1	NB
PIK3CA (Q546K)-PIK3R1	NB
PIK3CA-PIK3R1	ADP-Glo (US, Ger), NB
PIK3CA-PIK3R1/p65a	ADP-Glo (US)
PIK3CB (D1067A)-PIK3R1	ADP-Glo (Ger)
PIK3CB (D1067V)-PIK3R1	ADP-Glo (Ger)
PIK3CB (D1067Y)-PIK3R1	ADP-Glo (Ger)
PIK3CB (E1051K)-PIK3R1	ADP-Glo (Ger)
PIK3CB (E633K)-PIK3R1	ADP-Glo (Ger)
PIK3CB-PIK3R1	ADP-Glo (US, Ger), NB

Kinase	Assay Format
PIK3CD-PIK3R1	ADP-Glo (US, Ger), NB
PIK3CG	ADP-Glo (US, Ger)
PIK3CG (L1049R)-PIK3R1	ADP-Glo (Ger)
PIKFYVE	NB
PIM1	HS, PQ, CPA
PIM2	HS, PQ, CPA
PIM3	HS, PQ, NB, CPA
PIP4K2C	NB
PIP5K1A	ADP-Glo (US, Ger)
PIP5K1B	ADP-Glo (Ger), NB
PIP5K1C	ADP-Glo (US, Ger)
PKD2/PRKD2 (G848E)	HS
PKMYT1	HS, NB
PKN1	HS, PQ
PKN1-ANXA4 (Aex2Pex13)	HS
PKN1-TECR	HS
PKN2	HS, PQ
PKN3	HS, PQ
PLK1	HS, PQ, NB
PLK2	HS, PQ, NB
PLK3	HS, PQ, NB
PLK4	HS, PQ, NB
PNCK	HS
PRKAA1	PQ, NB
PRKAA1 (aa1-312)	PQ
PRKAA1-PRKAB1-PRKAG1	HS
PRKAA1-PRKAB1-PRKAG2	HS
PRKAA1-PRKAB1-PRKAG3	HS
PRKAA1-PRKAB2-PRKAG1	HS
PRKAA1-PRKAB2-PRKAG2	HS
PRKAA1-PRKAB2-PRKAG3	HS
PRKAA2	NB
PRKAA2-PRKAB1-PRKAG1	HS
PRKAA2-PRKAB1-PRKAG2	HS
PRKAA2-PRKAB1-PRKAG3	HS
PRKAA2-PRKAB2-PRKAG1	HS
PRKAA2-PRKAB2-PRKAG2	HS
PRKAA2-PRKAB2-PRKAG3	HS
PRKACA	HS, PQ, NB

Kinase	Assay Format
PRKACA (L206R)	HS
PRKACA-DNAJB1	HS
PRKACB	HS, NB
PRKACG	HS
PRKCA	HS, PQ
PRKCB (1)	HS, PQ
PRKCB (2)	HS, PQ
PRKCD	HS, PQ
PRKCE	HS, PQ, NB
PRKCG	HS, PQ
PRKCH	HS, PQ
PRKCI	HS, PQ
PRKCQ	HS, PQ
PRKCZ	HS, PQ
PRKCZ (aa184-592)	HS, PQ
PRKD1	HS, PQ
PRKD2	HS, PQ
PRKD2 (G870E)	HS
PRKD3	HS, PQ
PRKDC	HS, PQ
PRKG1 (A)	HS, PQ
PRKG1 (B)	HS
PRKG2	HS, PQ, NB
PRKX	HS, PQ, NB
PTK2	HS, PQ, NB, CPA
PTK2 (aa411-686)	PQ
PTK2B	HS, PQ, NB
PTK6	HS, PQ, NB
RAF1 (R391W)	HS
RAF1 (Y340/Y341D)	PQ
RAF1 (Y340D/Y341D)	HS
RET	HS, PQ, NB
RET (A883F)	HS
RET (E732K)	HS
RET (E762Q)	HS, PQ
RET (G691S)	HS, PQ
RET (G810C)	HS, PQ
RET (G810R)	HS, PQ
RET (G810S)	HS, PQ

Kinase	Assay Format
RET (L730I)	HS, PQ
RET (L730M)	HS, PQ
RET (L790F)	HS
RET (M918T)	HS, PQ, NB
RET (R749T)	HS, PQ
RET (R813Q)	HS, PQ
RET (R912P)	HS
RET (S891A)	HS, PQ
RET (S904A)	HS
RET (S904F)	HS
RET (V738A)	HS
RET (V778I)	HS
RET (V804E)	HS, PQ
RET (V804L)	HS, PQ, NB
RET (V804L)-KIF5B	HS
RET (V804M)	HS, PQ, NB
RET (V804M)-KIF5B	HS
RET (Y791F)	HS, PQ
RET (Y806C)	HS
RET (Y806H)	HS, PQ
RET (Y806N)	HS
RET-BCR	HS
RET-CCDC6	HS, PQ
RET-KIF5B	HS
RET-NCOA4	HS
RET-PRKARA1A	HS
RIOK2	NB
RIPK1	NB
RIPK2	HS, PQ, NB
RIPK3	HS
RIPK4	HS, PQ
ROCK1	HS, PQ, CPA
ROCK2	HS, PQ, CPA
ROS1	HS, PQ
ROS1 (G2032R)	HS
ROS1 (G2101A)	HS
ROS1 (G2101C)	HS
ROS1-GOPC	HS
ROS1-TPM3	HS

Kinase	Assay Format
RPS6KA1	HS, PQ, NB
RPS6KA2	HS, PQ, NB
RPS6KA3	HS, PQ, NB
RPS6KA3 (I416V)	HS, NB
RPS6KA3 (L608F)	HS, NB
RPS6KA4	HS, PQ, NB
RPS6KA5	HS, PQ
RPS6KA6	HS, PQ, NB
RPS6KB1	HS, PQ, CPA
RPS6KB2	HS, PQ
SBK1	HS
SBK3	NB
SGK1	HS, PQ, NB
SGK2	HS, PQ, NB
SGK3	HS, PQ
SIK1	HS, PQ, NB
SIK2	HS, PQ, NB
SIK3	HS, PQ, NB
SLK	HS, PQ, NB
SNRK	HS, NB
SPHK1	ADP-Glo (US)
SPHK2	ADP-Glo (US)
SRC	HS, PQ, NB, CPA
SRC (T341M)	HS
SRC (Y530F)	HS
SRMS	HS, PQ, NB
SRPK1	HS, PQ
SRPK2	HS, PQ
SRPK3	HS, PQ
STK3	HS, PQ, NB
STK4	HS, PQ, NB
STK10	HS, NB
STK11	HS, NB

Kinase	Assay Format
STK11-CAB39-STRADA	PQ
STK16	HS, PQ, NB
STK17A	HS, PQ
STK17B	PQ, NB
STK24	HS, PQ, NB
STK25	HS, PQ
STK26	HS, PQ, NB
STK32A	NB
STK32B	HS, NB
STK32C	HS
STK33	HS, PQ, NB
STK35	NB
STK36	NB
STK38	HS, PQ, NB
STK38L	HS, PQ, NB
STK39	HS, PQ
SYK	HS, PQ
SYK (aa356-635)	PQ
TAOK1	HS
TAOK2	HS, PQ
TAOK3	HS, PQ
TBK1	HS, PQ, NB
TEC	HS, PQ, NB
TEK	HS, PQ, NB, CPA
TEK (A1124V)	HS, NB
TEK (P883A)	HS, NB
TEK (R849W)	HS, PQ, NB
TEK (Y1108F)	HS, PQ, NB
TEK (Y897C)	HS, NB
TEK (Y897S)	HS, PQ, NB
TESK1	HS, NB
TESK2	HS
TGFBR1	HS, PQ

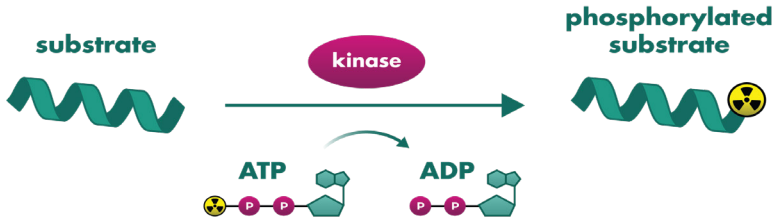
Kinase	Assay Format
TGFBR2	HS, PQ, NB
TIE1	NB
TIE2 (R915C)	HS
TIE2 (Y897H)	HS
TIE2 (Y897H/R915C)	HS
TLK1	HS, PQ, NB
TLK2	HS, PQ, NB
TNIK	HS
TNK1	HS, PQ, NB
TNK2	HS, PQ, NB
TNNI3K	NB
TRPM7	HS
TSSK1B	HS, PQ, NB
TSSK2	HS, PQ
TSSK3	HS
TSSK6	HS
TTBK1	HS, PQ
TTBK1 (aa1-480)	PQ
TTBK2	HS, PQ
TTK	HS, PQ, NB
TXK	HS, PQ, NB
TYK2	HS, PQ, NB
TYK2 (JH1)	NB
TYK2 (JH2)	NB
TYRO3	HS, PQ, NB
ULK1	HS, NB
ULK2	HS, PQ, NB
ULK3	HS, NB
VRK1	HS, PQ
VRK2	HS, PQ
WEE1	HS, PQ, NB
WEE2	NB
WNK1	HS, PQ
WNK2	HS, PQ
WNK3	HS, PQ
YES1	HS, PQ, NB
YES1 (T348I)	HS
ZAP70	HS, PQ
ZAP70 (Y319F)	HS

HS : Radiometric HotSpot Kinase Activity Assay
NB : NanoBRET Intracellular Kinase Assay
PQ : Radiometric ^{33}PanQinase Kinase Activity Assay
CPA : Cell Phosphorylation Assay
BaF3 : BaF3 Cell Proliferation Assay

Kinase Screening – Free Choice

With over 860+ kinases, Reaction Biology offers the largest selection of kinases available for screening and profiling services. It is the most used kinase screening service in the industry according to HTStec Kinase Profiling Trends Survey.

- Get the highest quality data possible with the gold standard radiometric assay format
- Any class of inhibitor can be tested including ATP competitive and non-competitive as well as allosteric inhibitors
- High-throughput compatible
- Customized assay development possible
- Deliverables: single concentration % inhibition; IC_{50} and/or Ki values
- A reference compound is included in every study for no additional cost



Assay formats

Compound screening on protein kinases is performed with highly sensitive radiometric assays. Phosphate from ^{33}P-labelled ATP is transferred onto a substrate and directly measured avoiding false positives and negatives common with other assay formats.

We offer two radiometric assay formats which differ only in the way of substrate retention via a filter membrane (HotSpot™ assay, used in the US facility) or on a ScintiPlate surface (^{33}PanQinase™ assay, used in the German facility).

Lipid kinases are screened with ADP-Glo Platform from Promega.

Kinase Panel Screening

Screen against the largest selection of kinases and the most widely used panel in industry. Our kinase panels are run once or twice per month allowing us to offer screening with a turnaround time of only two weeks.

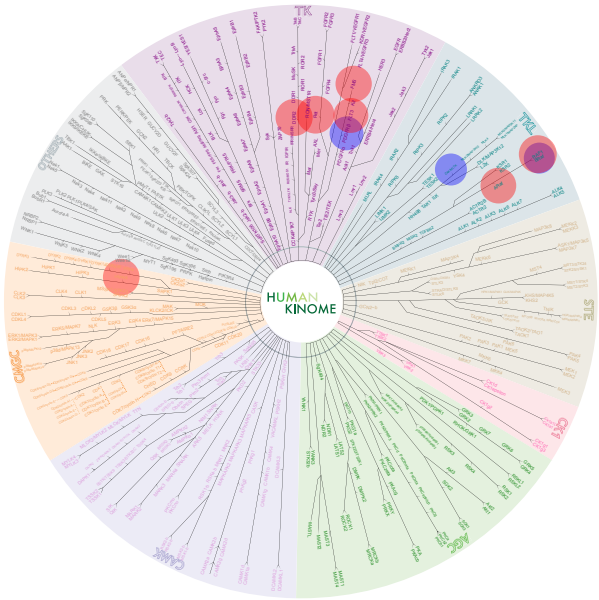
- Highest reproducibility
- Visualize your results with the kinase mapper
- Panels run with HotSpot™ assay include a free control compound's IC_{50} for every assay
- Deliverable: % of inhibition (single point) or IC_{50} value determination

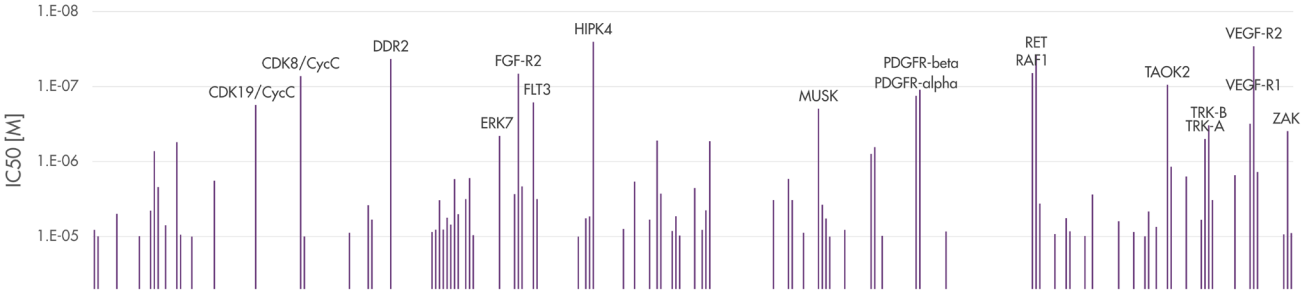
Panels run at US facility	# of kinases
Wild Type Kinase Panel	380
Mutant Kinase Panel	325
Atypical Kinase Panel	24
Lipid Kinase Panel	17
Diacylglycerol Kinase Panel	10

Panels run at German facility	# of kinases
IC50 Wild Type Kinase Panel	345
Wild Type Kinase Panel	345
Mutant Kinase Panel	96
Lipid Kinase Panel	14

Kinase Mapper

A kinase mapper tool can be used by customers for graphic presentation of kinase screening results. Shown is an example with Sorafenib profiled with the Wild Type Kinase Panel. Kinases that were inhibited more than 90% are highlighted in red circles, those inhibited more than 75% are shown in blue.

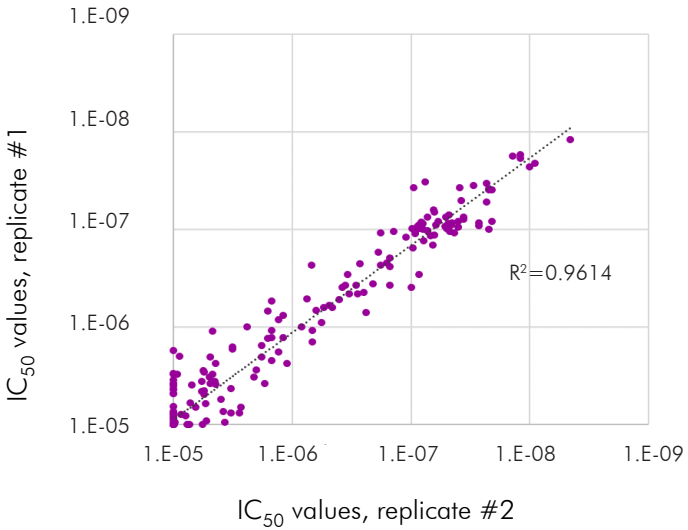




Example of IC_{50} value determination for sorafenib with the Wild Type Kinase Panel by using the ^{33}PanQinase™ assay format

Sorafenib activity was determined with 6 concentrations on 320 wild type protein kinases for IC_{50} value determination.

Using the IC_{50} value determination setup yields a true value of the inhibition of the compound for every individual kinase. False positives or false negatives that may occur when testing with a suboptimal concentration will be avoided.



High Reproducibility

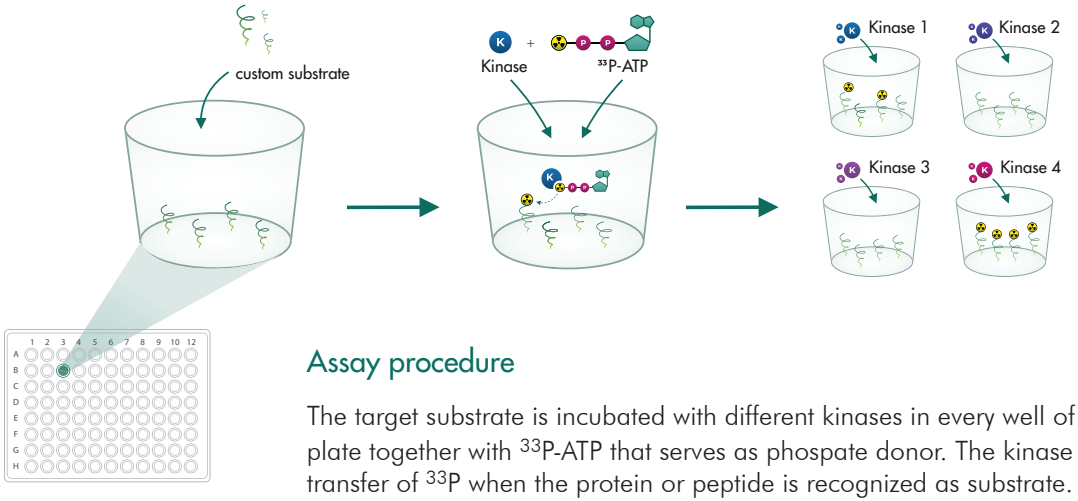
Selectivity profiles from two independent experiments with ponatinib on 320 wild type protein kinases.

KinaseFinder

The KinaseFinder can identify kinases that phosphorylate a substrate of interest. This service is ideal for the characterization of physiological pathways.

- Potential substrates can be peptides or proteins
- Option to compare peptides with phospho-site mutations
- Option to follow up with an SDS-PAGE and autoradiogram to visualize the phosphorylated substrate
- Deliverable: Absolute activity measurement of each kinase on your substrate

Type of KinaseFinder	# of kinases	Assay format
Tyr kinases	94	^{33}PanQinase™
Ser/Thr kinases	245	^{33}PanQinase™
Ser/Thr & Tyr kinases	339	^{33}PanQinase™
Wild Type Kinase Panel	380	HotSpot™
custom panel	selected by customer	HotSpot™



Assay procedure

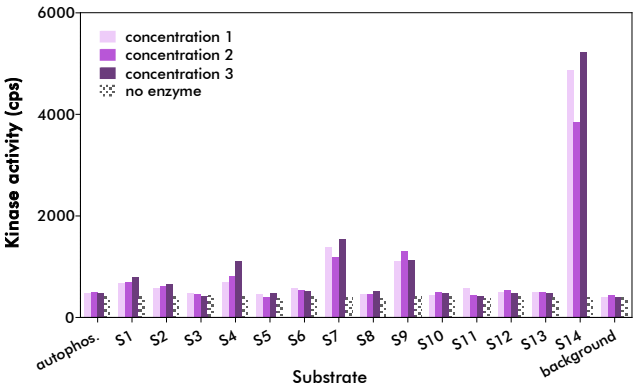
The target substrate is incubated with different kinases in every well of a multi-well plate together with ^{33}P-ATP that serves as phospate donor. The kinase will catalyse the transfer of ^{33}P when the protein or peptide is recognized as substrate. Quantification of phosphorylated substrate is performed via scintillation counting

Kinase SubstrateFinder

The Kinase SubstrateFinder can identify suitable substrates for a specific kinase of interest. The generic substrate panels comprise various proteins, whereas the physiological substrate panels include biotinylated peptide libraries.

- Testing of generic substrates with ATP consumption assay ADP-Glo (Promega)
- Testing of physiologic substrates with radiometric assay using ^{33}P-ATP
- Deliverable: absolute activity measurement of kinase with each substrate

Type of Kinase SubstrateFinder	# of substrates
Tyr Generic Substrate Panel	19
Ser/Thr Generic Substrate Panel	39
Ser/Thr & Tyr Generic Substrate Panel	58
Tyr Physiologic Substrate Panel	145
Ser/Thr & Tyr Physiologic Substrate Panel	720



Example of MELK assay development

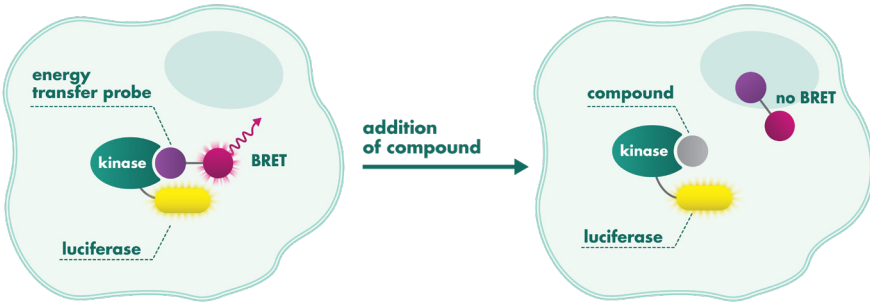
MELK kinase activity was measured with a variety of substrates. Controls are no-enzyme controls of each substrate and autophosphorylation of the MELK kinase without substrate.

Substrate 14 is to be most suitable for establishment of a MELK assay.

NanoBRET Intracellular Target Engagement Kinase Assay

Reaction Biology offers target engagement assays using Promega's NanoBRET technology that enables the quantitative determination of kinase inhibitor occupancy in live cells, without disruption of cellular membrane integrity.

- Intact cells with physiological ATP concentration, protein complex, co-factors and pH values.
- High-throughput compatible
- Deliverable: apparent binding affinity of inhibitor (IC_{50})

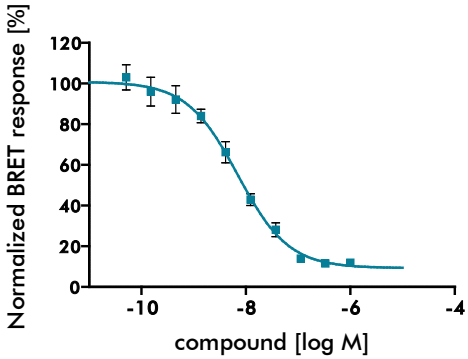


Assay principle

The assay is a compound competition assay that relies on bioluminescence resonance energy transfer (BRET) between a luciferase-tagged kinase and a fluorescent tracer. Quantitation and specificity are key attributes of the NanoBRET system.

DDR1 inhibition by Dasatinib

HEK293 cells transiently expressing NanoLuc®- DDR1 fusion vector were treated with the Tracer K-4 and reference compound Dasatinib for 1 hour. The BRET signal was measured on an EnVision 2104 multilabel microplate reader.



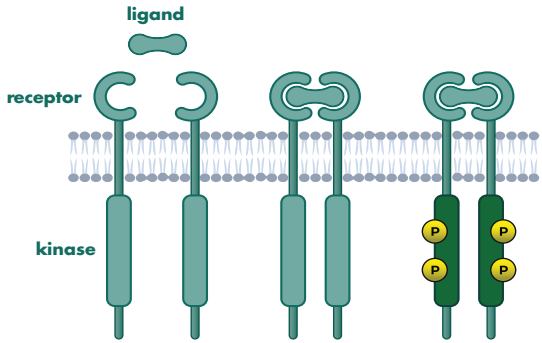
Cellular Phosphorylation Assay

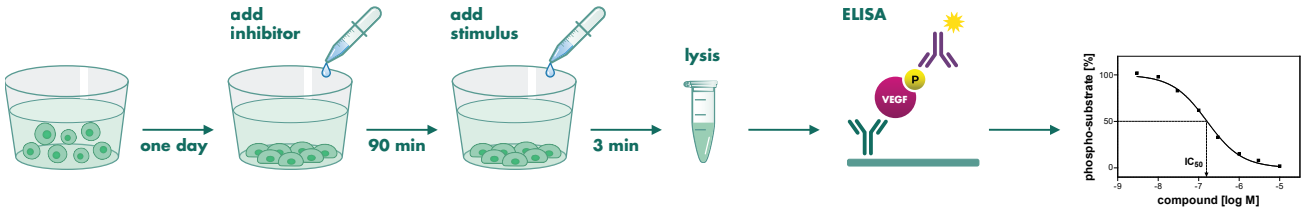
The Cellular Phosphorylation Assay quantifies changes in the phosphorylation state of a substrate as a result of treatment with your inhibitor in intact cells. The assays have been designed to address compound activity in a physiological environment on a physiological substrate.

- Physiological kinase, substrate and ATP concentrations
- Assay can be performed with blood containing drug for plasma-inhibitory study
- Deliverable: % inhibition of kinase activity and IC_{50} determination

Assays based on endogenous kinases

Assays are performed with cells expressing the kinase of interest which is either overexpressed or constitutively active, or kinase activity is triggered by ligand administration.



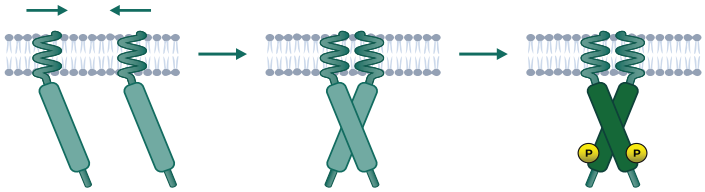


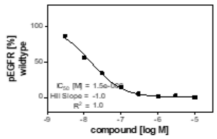
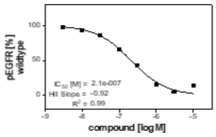
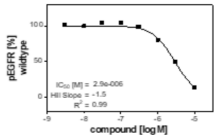
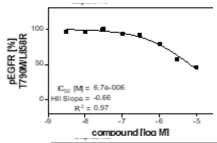
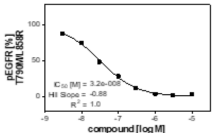
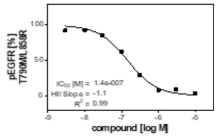
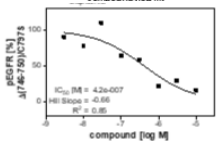
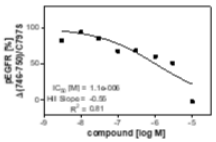
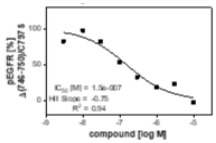
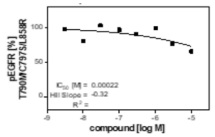
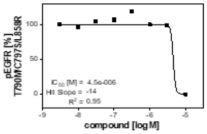
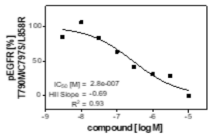
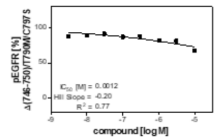
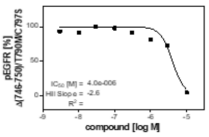
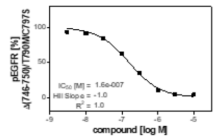
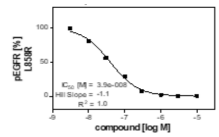
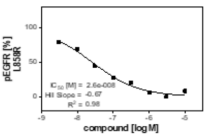
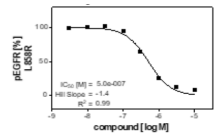
Example of VEGF-R2 signaling

Human endothelial cells are known to express VEGF-R2. The cells incubate with the test compound for 90 minutes to allow for target binding. After a 3-minute stimulation with ligand VEGF-A, cells are lysed and the substrate phosphorylation is quantified by ELISA with pan-phospho-tyrosine antibodies on captured VEGF-R2. The assays are performed with 8 compound concentrations in duplicate for IC50 value determination.

Assays based on exogeneous kinases

Rat 1 fibroblasts were transfected to stably express the intracellular domain of EGF-R mutants fused to an artificial transmembrane domain. Dimerization of the receptors causes constitutive auto-phosphorylation that can be quantified via ELISA.



	lapatinib	osimertinib	brigatinib
EGF-R Wild Type			
EGF-R T790M/L858R			
EGF-R Δ746750/C797S			
EGF-R T790M/C797S/L858R			
EGF-R Δ746-750/T790M/C797S			
EGF-R L858R			

Example of EGF-R mutant analysis

Rat1 fibroblasts express the intracellular domain of EGF-R containing disease-relevant mutations and a transmembrane domain. The cells were incubated with three EGF-R-specific inhibitors and their potency was quantified via ELISA.

BaF3 Cell Proliferation Assay

The BaF3 Cell Proliferation Assay is performed with BaF3 cells, a pro-B-cell line that is dependent on interleukin 3 for its survival and proliferation. Transgenic overexpression of oncogenic kinases, in particular receptor tyrosine kinases, can transform the cell line to become independent of interleukin 3. This tool cell line is suitable to investigate the potency of kinase oncogenes and the downstream effects of kinase inhibition.

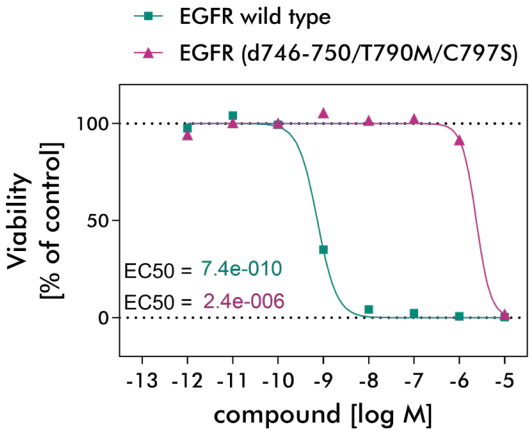
- Kinase inhibitor screening performed in the physiological environment of intact cells
- Determine the effects of compound treatment on the signaling activities of the target kinase
- Readout: Impact of kinase inhibition on cell proliferation

Available Assays

Target	Synonyms
EGFR wild type	ERBB, mENA, ERBB1
EGFR (d746-750/T790M/C797S)	ERBB, mENA, ERBB1

Study Example

BaF3 cells stably expressing EGFR wild type and EGFR (d746-750/T790M/C797S), respectively, were treated with kinase inhibitor Afatinib for 72 hours before quantification of live cells via Cell Titer Glo. The graph depicts the percentage of viable cells in relative to vehicle control (100 %) and staurosporine treatment (0 %).



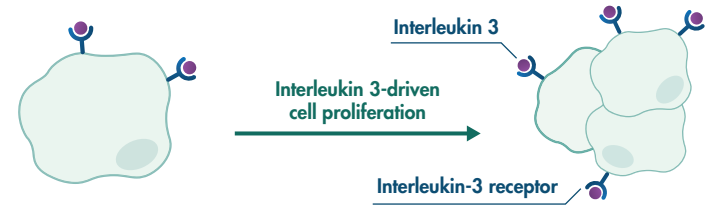
Assay Principle

The BaF3 cell line proliferates in the presence of interleukin 3.

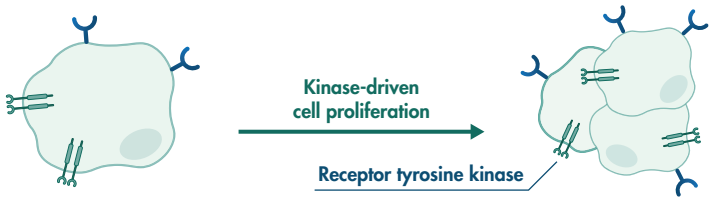
The overexpression of receptor tyrosine kinases enables the BaF3 cell line to grow without the supplement of interleukin 3. The cell growth is driven by the signaling of the kinase.

The inhibition of the activity of the transforming kinase leads to the loss of growth stimuli resulting in cell apoptosis.

A. Untransfected BaF3 cells



B. BaF3 cells transfected with kinase oncogene



C. Kinase oncogene inhibition

Assay Development

The BaF3 Cell Proliferation Assay can be performed with constructs of a large variety of receptor tyrosine kinases that can act as oncogenes driving cell survival, growth, and proliferation.

In Vivo Kinase Tumor Models

Genetically engineered tumor models are well suited for investigation of a single driver of tumor growth such as an overexpressed or constitutively expressed kinase.

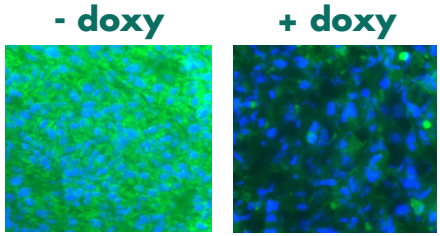
Reaction Biology offers two models based on fibroblast cells which were engineered to express an exogenous receptor kinase under the control of an inducible promotor. These models make excellent tools for the investigation of inhibitors in the in vivo setting.

- Target a human kinase in mice with intact immune system
- Implantation of engineered cells for comparable tumor growth
- Assess compound efficacy and evaluate mechanisms of drug resistance

Kinase	Cell line
human IGF receptor	MEF (mouse)
human ErbB2 receptor	NIH3T3 (mouse)

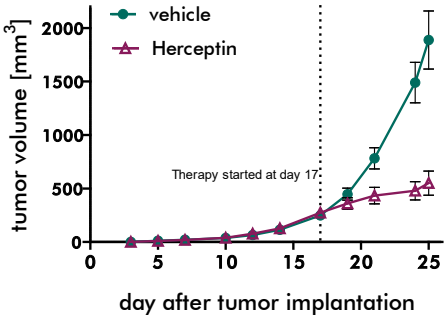
Example: ErbB2 Model

NIH3T3 cells were stably transfected to express human ErbB2 under the control of a Tet-inducible promoter. ErbB2 is expressed in the absence of doxycycline and expression is inhibited in the presence of doxycycline.



Example: ErbB2 Inhibition with Herceptin

NIH3T3-ErbB2-Rrep cells were implanted subcutaneously into mice. At an average tumor size of 400 mm^3, mice were treated with the anti-ErbB2 antibody Herceptin resulting in tumor regression.



Customized Kinase Drug Discovery

High-Throughput Screening

Bring your own compound library or use one of our libraries for high-throughput screening with our well validated kinase assays. Contact us to talk about the best approach for a successful screening project.

Custom Assay Development

Our experience of establishing more than 730 kinase assays is the basis for the successful development of the custom-tailored assay for your drug discovery project. We will be happy to provide guidance in construct selection, protein production, substrate requirements and assay condition optimization.

ATP and Substrate Competition Assay

To determine whether a compound's mechanism of action is ATP competitive or substrate competitive, we determine the IC_{50} or Ki values at various ATP and substrate concentrations.

Mechanism of Action Analysis

Using a variety of biochemical and biophysical methods, we can determine the kinetic behavior of your compound including binding affinity, residence time, on- and off-rates, that are crucial to your compound's therapeutic efficacy.

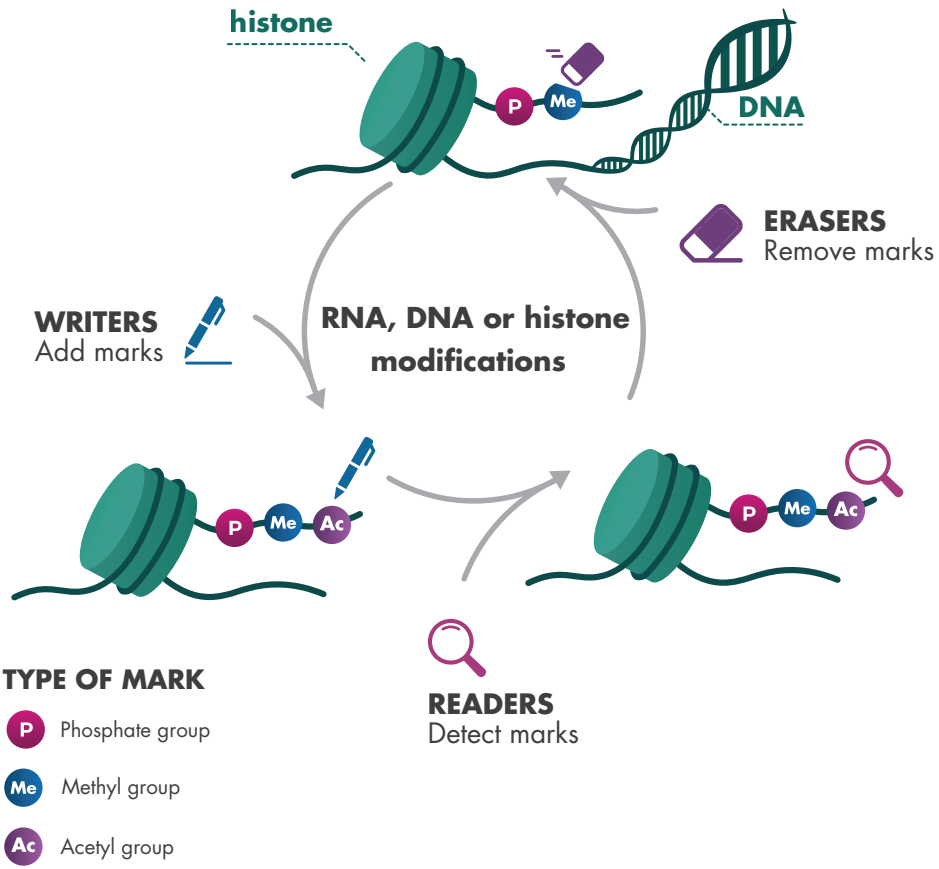
Kinetics, Binding affinity

SPR is commonly used to determine the kinetics of target-analyte binding kinetics. The assay detects changes in the molecular mass of a target after binding of the analyte. The target is immobilized to a sensor chip and the analyte flows to the target. Target binding is monitored in real-time for both: association and dissociation.

Kinase Activation Assay

The Kinase Activation Assay is suitable for the discovery of allosteric compounds that inhibit the activation of a target kinase by an upstream kinase in a so called cascade assay.

Epigenetic Assays



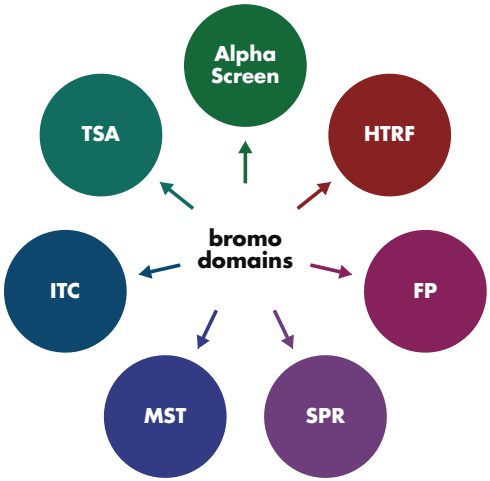
Reaction Biology offers extensive epigenetic drug discovery services including protein production, assay development, high-throughput screening, SAR support, mechanism of action analyses and cell-based assays.

The target families include proteins that regulate post-translational processes such as methylation, acetylation and phosphorylation.

Reader Domain Assays

Reaction Biology offers both biochemical and biophysical assays to study epigenetic reader domains. More than 100 assays have been established for screening, lead optimization or selectivity profiling for reader domain inhibitors.

- All reader domain proteins are produced at our facility and are available for purchase.
- Extensive coverage of the bromodomain family.
- Visualize your bromodomain profiling results with the mapper tool.



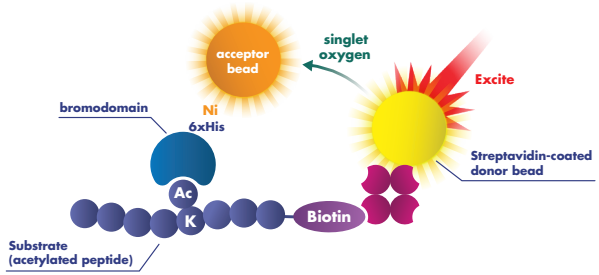
Assay formats available at Reaction Biology for bromodomain targets

Biochemical assay formats to quantify compound binding:

- AlphaScreen
- HTRF
- Fluorescence polarization (FP)

Biophysical assay formats for determination of binding affinity, on- and off-rates and parameters of agent-target interaction on the molecular level

- Surface plasmon resonance (SPR)
- Microscale thermophoresis (MST)
- Isothermal titration calorimetry (ITC)
- Thermal shift assay (TSA)



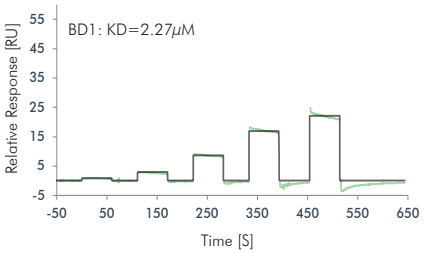
AlphaScreen assay

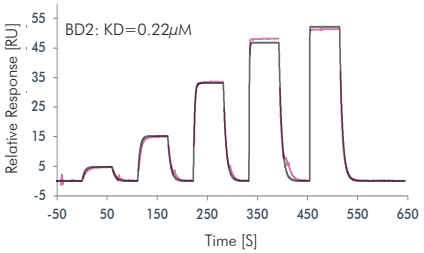
For bromodomain screening, an acetylated peptide substrate and a bromodomain protein are captured on AlphaScreen beads. A binding interaction between the substrate and protein brings the beads into close proximity. Laser excitation of the complex results in a sequence of chemical reactions resulting in an enhanced fluorescent emission. The presence of an inhibitor interferes with substrate/protein binding resulting in a reduced fluorescent signal. Reaction Biology offers AlphaScreen assays for many reader domains for screening and profiling.

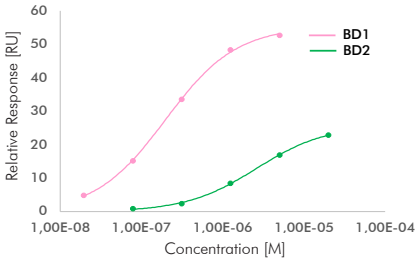
Surface plasmon resonance (SPR) assay

SPR measures biomolecular interactions in real time for screening of targets that are enzymes as well as non-enzymatic targets such as bromodomains. The target proteins are immobilized on the surface of a sensor chip. The compound flows over the sensor chip and binds to the target increasing the molecular mass of the protein upon binding which is measured with an optical readout.

Using SPR we can describe several parameters of the inhibitor-bromodomain interaction: 1. Which of my compounds bind? 2. Is the compound specific to my target? 3. How strong is the binding? 4. What are the association and dissociation rates? 5. Where does it bind?







Example of BRD4 domain interaction with RVX-208 as detected by SPR

Human bromodomain BRD4 contains tandem bromodomains (BD1 and BD2) that have unique biological functions. Inhibitors that selectively bind to one of the domains may affect different biological outcomes. By using recombinant BRD4 with individual bromodomains, SPR revealed that RVX-208 is about 10-times more selective for BD2 over BD1.

Reader Domain	Assays
ASH1L-[BRD]-GST	TS
ATAD2-His	TS, AS
ATAD2B-GST	TS
ATAD2B-His	TS, AS
BAZ1A-GST	TS
BAZ1B-His	TS, AS
BAZ2A-GST	TS
BAZ2A-His	AS
BAZ2B-His	TS, AS
BPTF-[BRD]-His	TS, AS
BPTF-[PHD-BRD]-His	TS, AS
BRD1-GST	TS
BRD1-His	TS, AS
BRD2-1-GST	TS
BRD2-1-His	TS, AS
BRD2-2-GST	TS
BRD2-2-His	TS, AS
BRD2-Tndm-His	TS, AS
BRD3-1-GST	TS
BRD3-1-His	TS, AS
BRD3-2-GST	TS
BRD3-2-His	TS, AS
BRD3-Tndm-GST	TS
BRD3-Tndm-His	TS, AS
BRD4 Full length	TS, AS
BRD4-1	SPR
BRD4-1-GST	TS
BRD4-1-His	TS, AS
BRD4-2	SPR
BRD4-2-GST	TS
BRD4-2-His	TS, AS
BRD4-Tndm-GST	TS
BRD4-Tndm-His	TS, AS
BRD7-GST	TS

Reader Domain	Assays
BRD9-GST	TS
BRD9-His	TS, AS
BRDT-1-His	TS, AS
BRDT-2-His	TS
BRDT-Tndm-His	TS, AS
BRPF1a	TS
BRPF1b-GST	TS
BRPF1b-His	TS, AS
BRPF3-GST	TS
BRPF3-His	TS, AS
BRWD1-2-GST	TS
BRWD1-2-His	TS, AS
BRWD3-2-GST	TS
CBX7-[CHR]-GST	TS
CDg1-[CHR]-GST	TS
CECR2-GST	TS
CECR2-His	TS, AS
CHD1-[CHR]-GST	TS
CHD1-[CHR]-His	TS
CHD2-[CHR]-GST	TS
CHD2-[CHR]-His	TS
CHD4-[CHR]-GST	TS
CHD4-[PHD-CHR]-GST	TS
CHD7-[CHR]-GST	TS
CREBBP-GST	TS
CREBBP-His	TS, AS
EED	HTRF
EP300-GST	TS
EP300-His	TS
HP1alpha-[CHR]-His	TS
HP1alpha-GST	TS
HP1beta-[CHR]-GST	TS
HP1beta-[CHR]-His	TS, AS

Reader Domain	Assays
HP1beta-GST	TS
HP1beta-His	TS, AS
HP1beta-Strep	TS
HP1gamma-GST	TS
HP1gamma-His	TS
KAT2A	TS
KAT2B	TS
KAT5-2-[CHR]-His	TS
KAT5-3-[CHR]-GST	TS
KAT5-3-[CHR]-His	TS
L3MBTL1	TS
L3MBTL1-His	AS
MPP8-[CHR]-GST	TS
PB1-1	TS
PB1-2	TS
PB1-3	TS
PB1-4	TS
PB1-5	TS
PB1-6	TS
PHIP-2	TS
PHIP-Tndm	TS
SMARCA2a bromodomain	HTRF
SMARCA2a-His	TS
SMARCA2b-His	TS, AS
SMARCA4-His	TS, AS
SP100-GST	TS
SP100-His	TS
SP110c-GST	TS
SP140-GST	TS
SP140-His	TS, AS
SP140L-GST	TS
SP140L-His	TS, AS
TAF1-1-GST	TS

Reader Domain	Assays
TAF1-2-GST	TS
TAF1-2-His	AS
TAF1L-1	TS
TAF1L-2	TS
TAF1L-Tndm-GST	TS
TRIM24	TS
TRIM28	TS
TRIM33a	TS
TRIM33b-His	TS
TRIM66	TS
UHRF1 Full length	TS
UHRF1-[PHD]	TS
UHRF1-[PHD]-His	AS
UHRF1-[SRA]	TS
UHRF1-[TDR-PHD]	TS
UHRF1-[TDR-PHD]-His	AS
UHRF1-[TDR]-His	TS, AS
UHRF1-His Full length	AS

AS...
AlphaScreen

TS....
Thermal Shift Assay

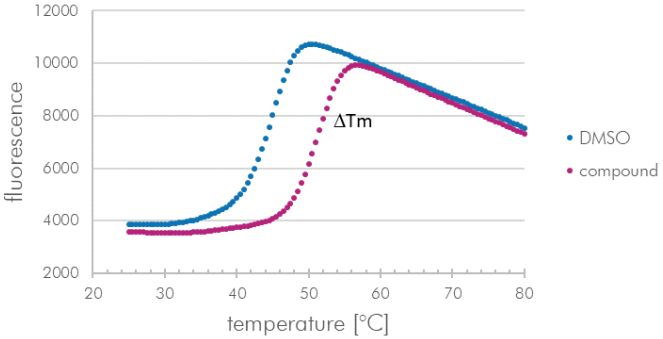
HTRF .....
Homogenous Time-Resolved Fluorescence

SPR .....
Surface Plasmon Resonance

BromoMELT

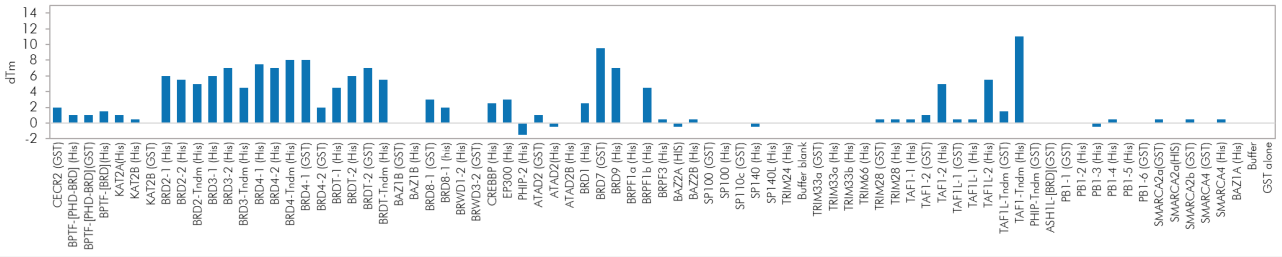
BromoMELT is a thermal shift assay for bromodomain targets that measures the change in protein melting temperature upon the binding of a ligand. Protein melting measurements are useful for identifying ligands, buffer conditions, co-factors and drugs affecting protein stability.

- Available as service or as kit to easily perform the assay in your own lab using a qPCR machine
- Includes 77 proteins representing 63 bromodomains
- Any inhibitor can be characterized within hours
- High-throughput compatible



Assay principle

The thermal shift assay determines the melting temperature at which there is 50% denaturation of the target protein. The difference between the melting temperatures of protein only (blue line) and protein plus ligand (purple line) is proportional to the binding affinity of the interaction.



Example of the selectivity profile of bromosporine

The binding of bromosporine to 77 bromodomain proteins was characterized using the BromoMELT assay kit. The difference in melting temperatures of target proteins bound to bromosporine versus DMSO control is proportional to the binding affinity of the protein/bromosporine interaction.

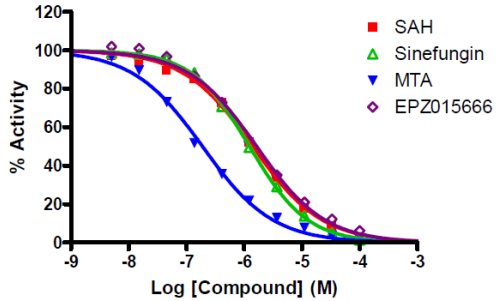
Methyltransferase Assays

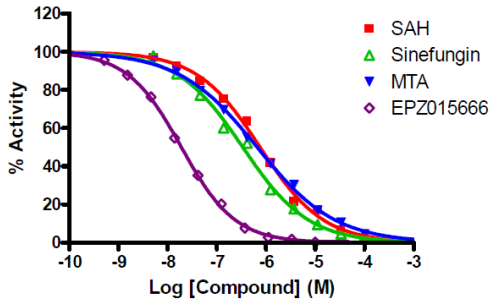
Reaction Biology offers radiometric activity assays and recombinant proteins for over 30 methyltransferases.

- Direct measurement of enzyme activity via radiometric assay
- Detection of inhibitors with varying binding modes
- Substrates can be nucleosomes, histones, peptides or other substrates
- Deliverable: % inhibition (single or multiple concentration) or IC_{50} values

Assay principle

Methyltransferases use tritium-labeled S-adenosyl-L-methionine (SAM) as the methyl donor that is converted to S-adenosyl-L-homocysteine (SAH) during the transfer of the radioactive methyl group to the histone substrate.





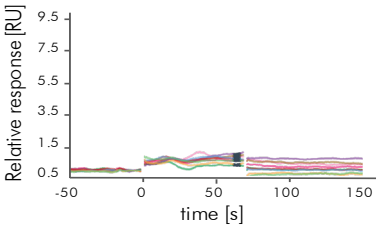
Example: Inhibition of PRMT5/MEP50 activity

Concentration-dependent inhibition of PRMT5/MEP50 activity by inhibitors in comparison to SAH on either histone HA2 substrate (HotSpot assay, left) or H4-biotin substrate (FlashPlate assay, right).

Biophysical assays such as surface plasmon resonance (SPR) can be used to determine the binding affinities of inhibitors to epigenetic targets including enzymes and non-enzymatic proteins.

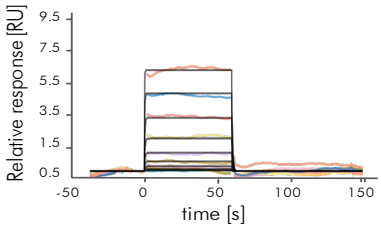
no co-factor

KD = no binding



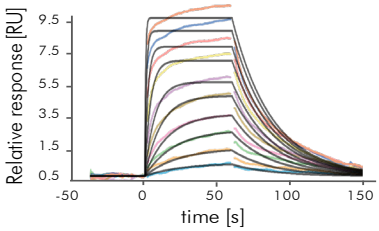
presence of MTA

KD = 2.1×10^{-5}M



presence of SAH

KD = 6.6×10^{-7}M



Example of a co-factor analysis by SPR

EPZ015666 is a substrate-competitive inhibitor that binds to its target PRMT5/MEP50 only in the presence of SAM or SAM analogues such as MTA and SAH.

Methyltransferase	Protein available
ASH1L	√
COMT	√
COMT (V108M)	√
DNMT1	√
DNMT3a	√
DNMT3b	√
DNMT3b/DNMT3L	√
DOT1L	√
EZH1 Complex	√
EZH2 (Y641F) Complex	√
EZH2 Complex	√
G9a	√
GLP	√
METTL21A	-
METTL3/METTL14	-

Methyltransferase	Protein available
MLL1 Complex	√
MLL2 Complex	√
MLL3 Complex	√
MLL4 Complex	√
NRMT1	√
NSD1	√
NSD2	√
NSD2 (E1099K)	√
NSD2 (T1150A)	√
NSD3	√
PRDM9	√
PRMT1	√
PRMT3	√
PRMT4	√
PRMT5 (C449S)/ MEP50	√

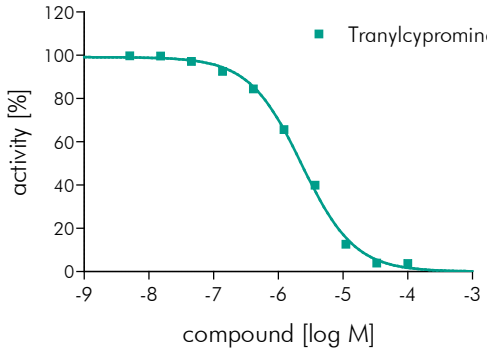
Methyltransferase	Protein available
PRMT5/MEP50	√
PRMT6	√
PRMT7	√
PRMT8	√
SET1B	√
SET7	√
SET8	√
SETD2	√
SETDB1	√
SMYD2	√
SMYD3	√
SUV39H1	√
SUV39H2	√
SUV420H1-tv2	√

Demethylase Assays (KDM)

Reaction Biology offers assays for both Jumonji C-domain containing (JmjCs) and lysine-specific demethylases (LSD) histone demethylase subfamilies. LSDs are flavin-dependent monoamine oxidases that catalyze demethylation of Kme2 or Kme1 producing peroxide (H_2O_2) and formaldehyde (H_2CO) in the process. JmjC are Fe(II)/2-oxoglutarate-dependent dioxygenases that use a reactive Fe(IV)-oxoferryl species to catalyze hydroxylation reactions.

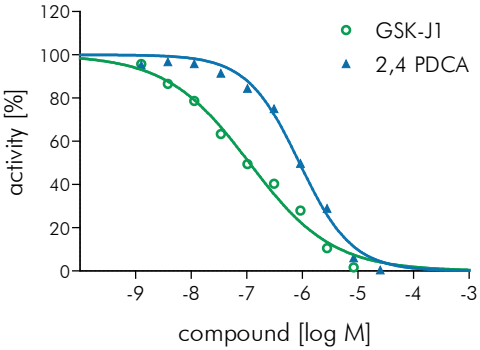
- Three assay formats are available to measure the activity of demethylases
- Customized conditions are available
- Deliverable: % inhibition (single or multiple test concentrations) or IC_{50} determination

Demethylase	Assay format	Protein available
KDM4A	HTRF	√
KDM4D	AlphaLISA	-
KDM5A	HTRF	√
KDM5B	HTRF	√
KDM5C	HTRF	√
KDM6B	AlphaLISA	√
LSD1	Amplex Red	√



Example of LSD1 inhibition

LSDs activity was detected by quantification of H_2O_2 using Amplex Red reagent for IC_{50} value determination of a reference inhibitior.



Example of KDM5C inhibition

KDM5C activity was detected using HTRF (homogeneous time resolved fluorescence) technology with KDM5C and substrate-specific antibody for IC_{50} value determination of two reference inhibitors.

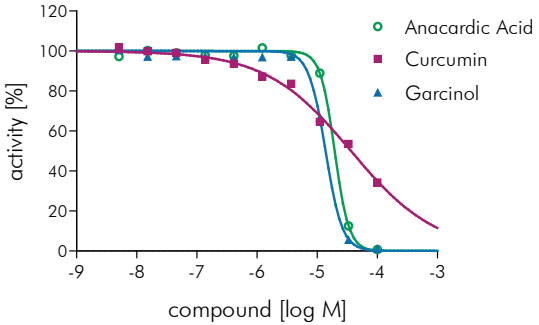
Histone Acetyltransferase Assays (HAT)

Reaction Biology offers radiometric activity assays for HAT enzymes using tritiated acetyl-Coenzyme A as cofactor.

- Direct measurement of enzyme activity via radiometric assay
- Customized conditions are available
- Deliverable: % inhibition (single or multiple test concentrations) or IC_{50} determination

Assay principle

Histone acetyltransferases acetylate lysines on histones and other proteins using tritium-labelled acetyl-Coenzyme A as the acetyl donor. The tritium-acetyl group is transfered onto histone substrate that is measured directly to reflect the enzyme activity.



Example of CBP inhibition

Full concentration-response of three reference inhibitors against CREP-binding protein CBP activity.

HAT	Protein available
CBP	√
KAT2A	√
KAT2B	√
KAT5	√
KAT6A	-
KAT6B	-
KAT7	-
KAT8	-
p300	√

Histone Deacetylase (HDAC) and Sirtuin Assays

Reaction Biology offers fluorescence-based assays for both Zn^{2+}-dependent HDACs and NAD^{+}-dependent sirtuins.

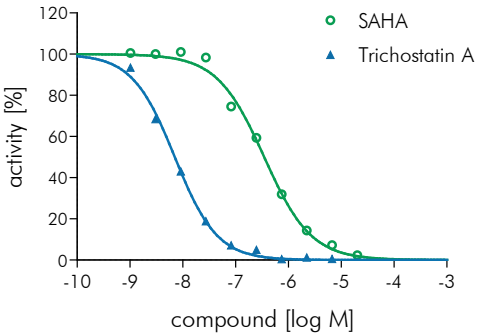
Each assay is optimized based on its specific substrate:

- HDAC 1, 2, 3, 6, 10, and Sirt 1, 2, 3: p53 residues 379-382 (RHKKAc)
- HDAC 8: p53 residues 379-382 (RHKAcKAc)
- HDAC 4, 5, 7, 9, 11: Trifluoroacetyl lysine
- Sirt 5: Ac-Lys(Succinyl)-AMC

- Customized conditions and kinetic studies are available
- Deliverable: % inhibition (single or multiple test concentrations) or IC_{50} determination

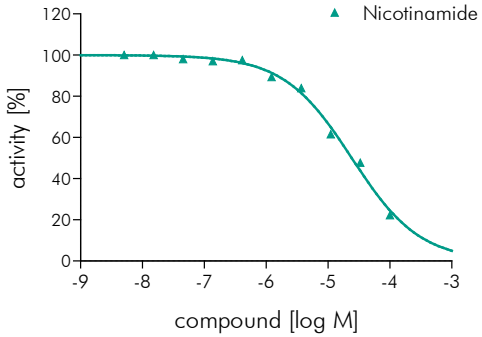
Assay principle

The assay is performed with purified human protein and a fluorigenic acetylated peptide substrate specifically designed for each enzyme. The deacetylated fluorigenic substrate is susceptible to cleavage by a protease to yield fluorecence.



Example of HDAC1 inhibition

Full concentration-response of two reference inhibitors against HDAC1 activity.



Example of SIRT5 inhibition

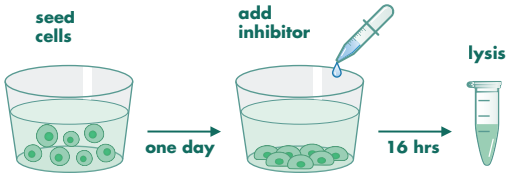
Full concentration-response of Nicotinamide, a pan-SIRT inhibitor, against SIRT5 activity.

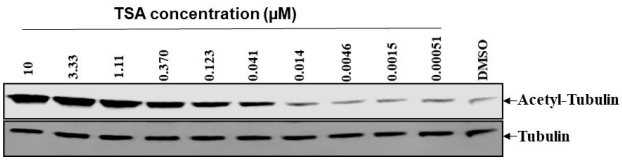
Cell-based Epigenetic Assays

Cell-based assays are valuable tools for evaluating inhibitor potency to affect acetylation and/or methylation changes of substrates in a physiological environment using intact cells. The detection options include ELISA, Western Blot, NanoBRET and HDAC-Glo assays.

- Evaluate compound activity in intact cells.
- ELISA and Western Blot detect the endogenous substrates for direct activity measurement
- Deliverable: IC_{50} values of epigenetic enzyme inhibition

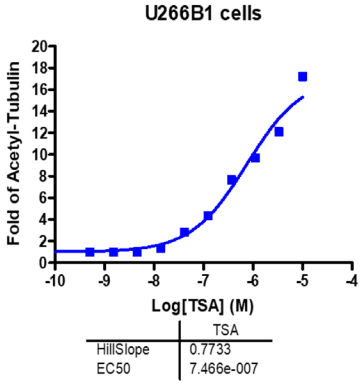
Readout	Assay format
Histone & Tubulin Deacetylation	Western Blot; ELISA
Histone Deacetylation (Class I/II)	HDAC-Glo
Histone Deacetylation (Class IIa)	HDAC-Glo
Histone Methylation	Western Blot
Histone Phosphorylation	ELISA





Example for detection of HDAC activity in cancer cells

Lysates of U266B1 cells treated with Trichostatin A (TSA) were subjected to Western Blotting and quantification via infrared imaging. The results are plotted in a curve for EC50 determination.



Ras Pathway Assays

Reaction Biology provides a variety of services to discover new inhibitors targeting the RAS pathway. The small GTPase, RAS, is a known oncogene that is mutated in a large percentage of cancers and is associated with poor disease prognosis. Mutated RAS is locked in the activated GTP bound state and facilitates enhanced RAS signaling in cancer cells.

Most of our assays are available with wildtype and mutated RAS variants.

Available Assay Formats	Description
Nucleotide Exchange Assay	Measuring of SOS1/2 mediated exchange of fluorescently labeled GDP to GTP Alternative readout: Observation of an increase in HTRF upon binding of fluorescent GTP to K-RAS
Protein-Protein Interaction of RAS and SOS1	HTRF based assay for testing of compounds that disrupt SOS1 binding to RAS.
Protein-Protein Interaction of RAS and cRAF	HTRF-based assay for testing of compounds that disrupt cRAF binding to RAS. This assay is also suited for quantification of nucleotide exchange reaction.
Thermal Shift Direct Binding Assay	Compound binding affinity measurement suited for measurement of compound selectivity to RAS mutant panel.
SPR Direct Binding Assay	Surface Plasmon Resonance (SPR) determines the kinetics of compounds binding RAS and RAS mutants or SOS.
NanoBRET Target Engagement RAS Assay	Intracellular measurement of the binding affinity of compounds via competitive displacement of a switch I/II pocket tracer

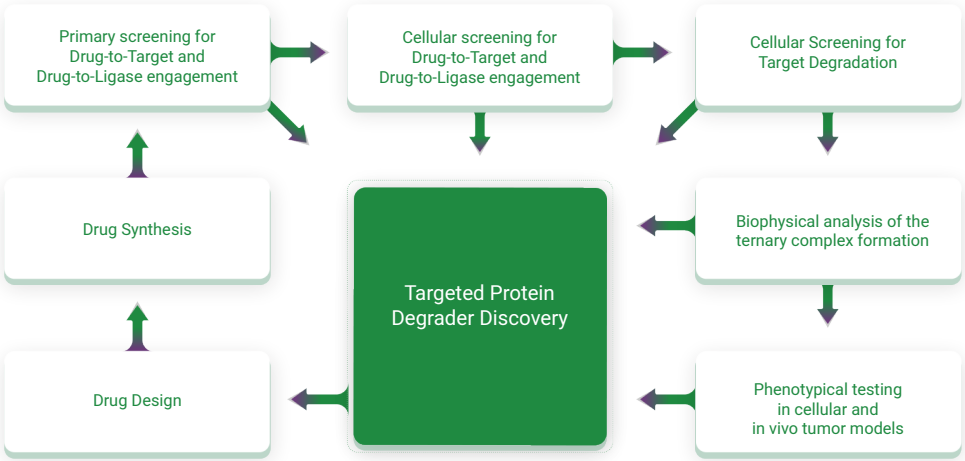
In addition to RAS specific assays we offer more than 80 RAS Pathway related assays for testing inhibitors against the ERK/MAPK and the PI3K signaling pathway as well as upstream pathways such as EGFR signaling including kinases, phosphatases and transcription factors.

Targeted Protein Degradation Assays

Rethinking PROTAC: An AI based platform to support your drug discovery project

Together with Medinoah, a medicinal chemistry provider, and PMRBioinfo, an AI computational company, we have created the Targeted Protein Degradation drug discovery platform. Medinoah has been one of the first CROs in synthesizing protein degradation molecules and advanced two PROTAC molecules into the IND stage. The AI powered Targeted Protein Degradation discovery platform will enable prediction of the binding mode of the ternary complex to significantly reduce the number of compounds and screening cycles needed for the generation of potent and optimized protein degradation molecules.

Workflow of discovery of new Targeted Protein Degradation molecules platform:

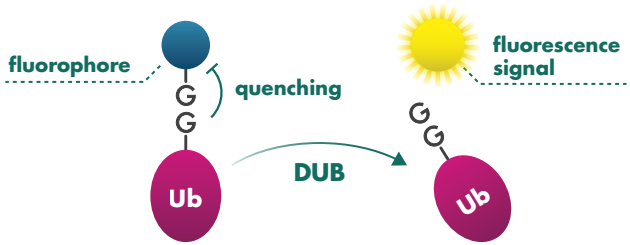


We are currently talking to biotech and pharma companies to perform fee-for-service, shared-cost collaboration or FTE-based work for the development of new Targeted Protein Degradation molecules. Please reach out to us to discuss options for supporting your Targeted Protein Degradation drug discovery program.

Ubiquitin-Proteasome Pathway Assays

Deubiquitinase enzymes (DUBs) are a group of proteases that cleave ubiquitin from proteins and other molecules. Ubiquitination of proteins affects protein degradation, cellular location, activities and protein-protein interactions. Reaction Biology offers fluorescent-based assays for screening of DUB inhibitors.

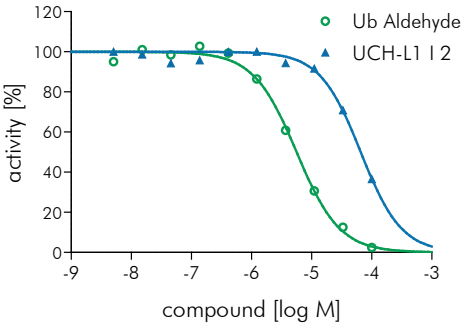
- Fluorescence-based quantification of deubiquitinase and 20S proteasome activity
- Custom assay development
- Deliverable: % inhibition (single point) or IC_{50} profiling, Ki determination



Available Targets
20S Proteasome
A20
Ataxin3
BAP1
NEDP1
SENP1
SENP2
UCHL1
UCHL3
USP2
USP4
USP5
USP7
USP8
USP10
USP11
USP14

Assay procedure

Fluorescent Ubiquitin-AMC is a substrate containing the fluorophore, 7-amido-4-methylcoumarin (AMC), that is quenched when ubiquitinylated. Upon incubation with a deubiquitinase, AMC is released and it's fluorescence can be measured.



Example of A20/TNFAIP3 inhibition

Concentration-dependent inhibition of deubiquitinase A20 by two inhibitors. IC_{50} value determination is based on 10 compound concentrations.

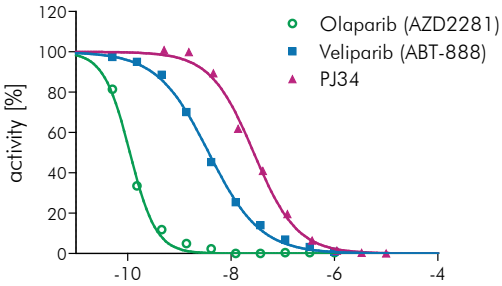
PARP Assays

Poly (ADP-ribose) polymerase (PARP) is a family of proteins that transfer ADP-ribose units from NAD+ onto target nuclear proteins forming long branched Poly ADP-ribose chains. PARPs play a role in epigenetic regulation, for example, by poly ADP-ribosylation of histone substrates.

- Gold standard assay format: radiometric activity assay
- High-throughput compatible
- Custom assay development
- Deliverable: % inhibition (single point) or IC_{50} profiling

Assay procedure

Adenylate-NAD^+ serves as co-factor for transfer of ^{32}P-labelled ADP-ribose units onto histones which will be quantified via scintillation counting.



Example of PARP2 inhibition

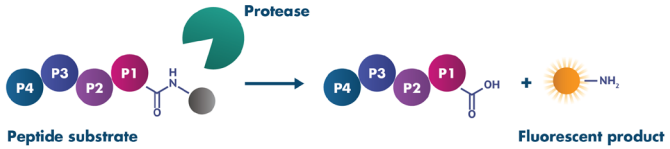
Dose-dependent inhibition of PARP2 by three reference inhibitors. IC_{50} value determination is based on 10 compound concentrations.

Protease Assays

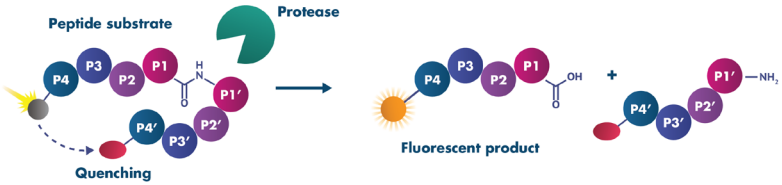
Reaction Biology offers a 65-member protease panel for routine profiling. Members include cysteine proteases, serine proteases, metalloproteases, aspartyl proteases, dipeptidases and others. Over 80 proteases are available for customized orders.

- Fluorescence-based activity assay
- High-throughput compatible
- Custom-assay development
- Deliverable: % inhibition (single point) or IC_{50} value determination

Fluorogenic peptide substrate



FRET/quencher peptide substrate



Assay formats

Upper image: Fluorogenic peptide substrate is cleaved by target protease releasing a fluorescent product.

Lower image: The peptide substrates contain a fluorophore and a quencher suppressing fluorescence unless substrate is cleaved.

Proteases		
ACE1	Cathepsin S	MMP 3
ACE2	Cathepsin V	MMP 7
Activated Protein C	Chymase	MMP 8
ADAM10	Chymotrypsin	MMP 9
BACE1	DDP-IV	MMP 10
Calpain1	DPP-IX	MMP 12
Caspase 1	DPP-VIII	MMP 13
Caspase 2	Elastase	MMP 14
Caspase 3	Factor VIIa	Neprilysin
Caspase 4	Factor Xa	Papain
Caspase 5	Factor XIa	Plasma Kallikrein
Caspase 6	Factor XIIa	Plasmin
Caspase 7	Furin	Proteinase A
Caspase 8	Granzyme B	Proteinase K
Caspase 9	Hepsin	SARS-CoV-2 Mpro
Caspase 10	HIV-1	SARS-CoV-2 PLpro
Caspase 11	Kallikrein 1	TACE
Caspase 14	Kallikrein 2	Thrombin
Cathepsin B	Kallikrein 5	TMPRSS11D
Cathepsin C	Kallikrein 7	TMPRSS2
Cathepsin D	Kallikrein 12	tPA
Cathepsin E	Kallikrein 13	Trypsin
Cathepsin G	Kallikrein 14	Tryptase g1
Cathepsin H	Matriptase 2	Urokinase
Cathepsin K	MMP 1	
Cathepsin L	MMP 2	

Phosphatase Assays

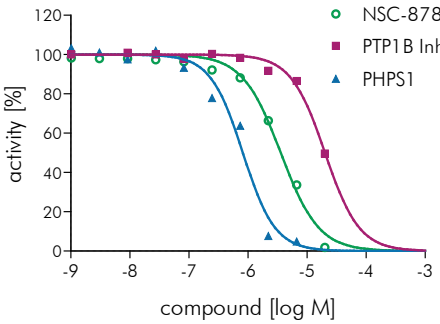
Protein phosphatases play important roles in cell signalling processes in interplay with kinases. Different than kinases, phosphatases are less specific for their substrates. All phosphatases catalyze the same basic hydrolysis reaction.

- Fluorescence-based activity assay
- High-throughput compatible
- Custom-assay development
- Deliverable: % inhibition (single point) or IC_{50} value determination

Phosphatases
DUSP22/MKPX
PP1A
PP1B
PP2A alpha/PPP2R1A Complex
PPAC (ACP1/LMW-PTP-A)
PPAC (ACP1/LMW-PTP-B)
PTPN1/PTP1B-CD
PTPN1/PTP1B-FL
PTPN2/TC-PTP
PTPN6/SHP1
PTPN7/LC-PTP
PTPN11/SHP2 (E76K)-FL
PTPN11/SHP2-CD
PTPN11/SHP2-FL
PTPN12/PTP-PEST
PTPRB
PTPRC/CD45
PTPRJ/CD148

DiFMUP-based assay principle

The fluorinated MUP derivate is suitable as substrate for a large range of protein phosphatases. The reaction product of DiFMUP is fluorescent after dephosphorylation.



Example of PTPRC/CD45 inhibition

Full concentration-response of 3 reference inhibitors.

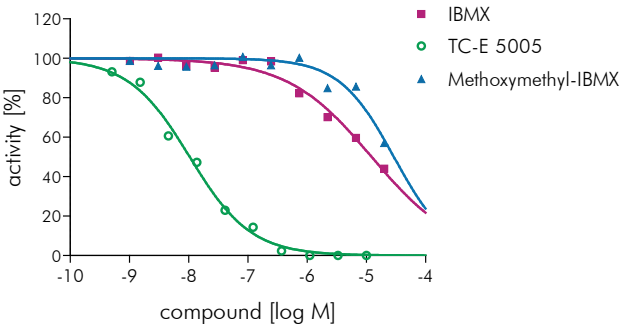
Phosphodiesterase (PDE) Assays

Phosphodiesterases (PDEs) catalyze the hydrolysis of cyclic AMP and cyclic GMP, thereby regulating these cyclic nuleotides' signaling pathways. PDE inhibitors have found utility in the treatment of a variety of conditions including pulmonary hypertension, acute refratory cardic failure, erectile dysfunction, etc.

- Activity of enzymes is measured with the Transcreeneer AMP2/GMP2 FP PDE assay platform (BellBrook labs)
- High-throughput compatible
- Custom-assay development
- Deliverable: % inhibition (single point) or IC_{50} values

Assay principle

PDE converts cAMP or cGMP to AMP or GMP which displace a fluorescent tracer from an antibody selective for AMP and GMP resulting in reduction of the fluorescence polarization signal.



Full concentration-response for three reference inhibitors of PDE 10A.

PDEs
PDE10A
PDE1A
PDE1B
PDE1C
PDE2A
PDE3A
PDE3B
PDE4A
PDE4B
PDE4C
PDE4D
PDE5A
PDE7A
PDE8A
PDE9A

Metabolic Pathway Assays

Acetyl-CoA Carboxylase (ACC)

ACC is a biotin-dependent enzyme that catalyzes the ATP-dependent carboxylation of acetyl-CoA to malonyl-CoA. ACC is a crucial metabolic enzyme and attractive drug target. Reaction Biology provides compound screening against ACC by detecting the production of ADP.

Carboxylase
ACC1
ACC2

Isocitrate dehydrogenase - IDH

Isocitrate dehydrogenases 1 and 2 (IDH1 and IDH2) are key metabolic enzymes that catalyze the conversion of isocitrate to a-ketoglutarate (aKG) and co-factor NADPH. Reaction Biology provides compound screening against IDH by measuring enzyme activity in a coupled system wherein NADPH produced in the initial reaction is a co-factor in the conversion of resazurin to fluoresecent resorufin in a secondary reaction.

IDHs are also available for purchase.

IDH	
IDH1 G97D	IDH2 R140K
IDH1 R100A	IDH2 R140Q
IDH1 R100Q	IDH2 R172Q
IDH1 R132C	IDH2 WT
IDH1 R132H	
IDH1 Y139D	
IDH1 WT	

NAD(P)H dehydrogenase [quinone] 1 - NQO

NQOs are involved in detoxification and biosynthetic pathways. Reaction Biology provides compound screening against NQOs by monitoring enzyme activity in an analogous coupled reaction as descripbed above for IDHs.

NQO
NQO1

Nucleotide Metabolism Pathway Assays

Nucleotide metabolism is the process in which nucleic acids (RNA, DNA, and cellular bioenergetics) are synthesized and degraded. DHODH (dihydroorotate dehydrogenase) synthesizes orotate from dihydroorotate (DHO) in the de novo pyrimidine synthesis pathway. The enzyme was shown to indude the differentiation of acute myeloide lymphomas and is therefore interesting as a drug target.

DHODH
DHODH
dDHODH (dog)
mDHODH (mouse)
rDHODH (rat)

Ion Channel Assays

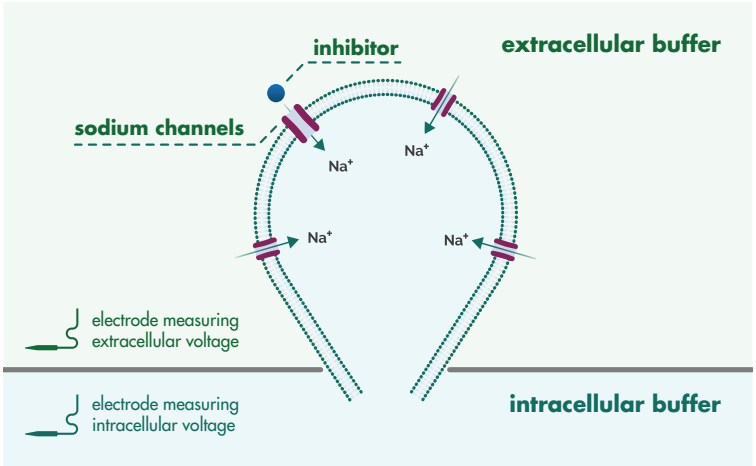
Reaction Biology offers cell-based ion channel testing for drug discovery and evaluation of drug safety.

- Cardiac-safety panel is available for pre-clinical evaluation of compound safety
- Three formats: two electrode-voltage clamp, QPatch and manual patch clamp
- Investigation of voltage-gated and ligand-gated ion channels
- Deliverable: IC_{50} value of inhibition of ion channel activity

Q Patch assay

QPatch HTX and QPatch 16 are automated patch clamp platforms that allow for the testing of up to 48 or 16 cells in parallel, respectively. Both systems provide whole-cell patch clamp data based on true gigaohm seals.

Ion channels	
hERG	Q Patch, Manual Patch Clamp
hNav1.5	Q Patch, Manual Patch Clamp
hCav1.2	Q Patch, Manual Patch Clamp

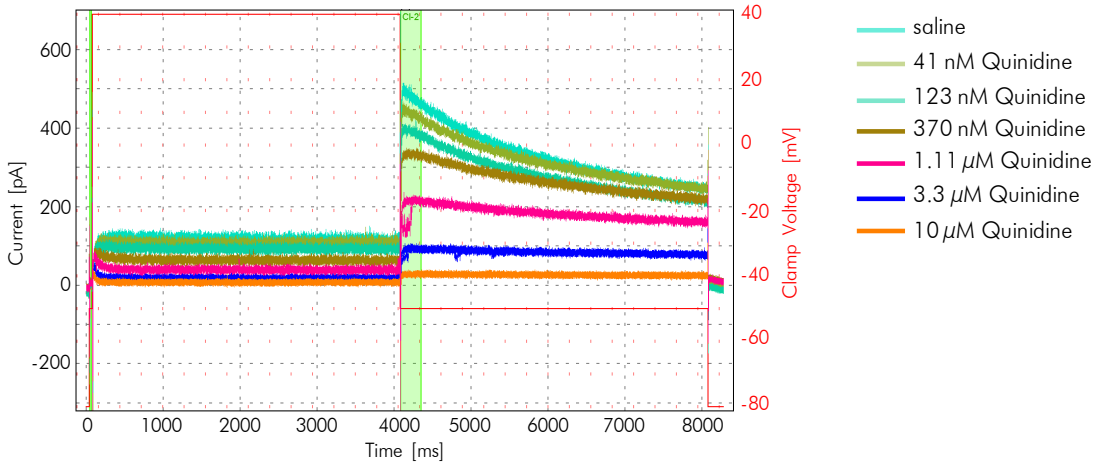


Assay principle

Schematic of a cell expressing sodium channels in a planar patch-clamp setup with recording in the whole-cell format.

Manual Patch Clamp Assay

Manual patch clamping is the gold standard for the investigation of ion channel activity. In addition to confirming the activity of preliminary actives from screens, manual patch-clamping can be used to assess mechanism of action of compounds and to determine the effects of compounds on the biophysical properties of a channel.



Example of hERG inhibition by Quinidine

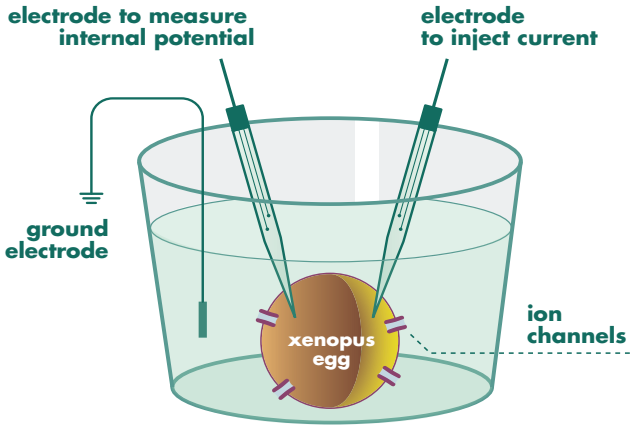
hERG potassium channel inhibition in the presence of various concentrations of Quinidine. Recordings were made on the QPatch in CHO cells stably expressing hERG voltage-dependent potassium channel. Each concentration of Quinidine was perfused for 5 minutes. 10µM Quinidine shows close to complete inhibition of hERG.

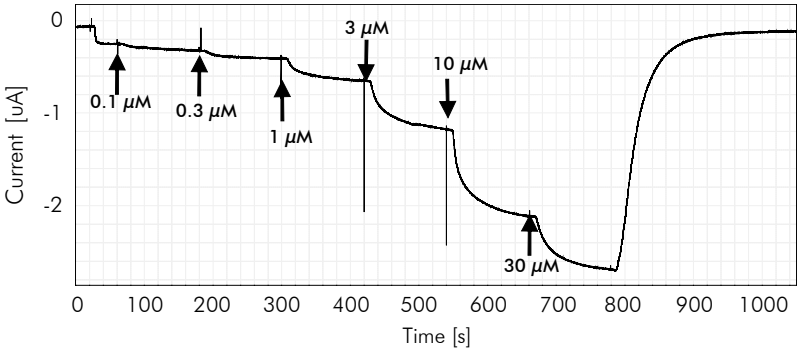
Two-Electrode Voltage Clamp

Two-electrode voltage clamps uses Xenopus laevis oocytes to test the activity of inhibitors against voltage-gated or ligand-gated ion channels. Ion channels of interest, and mutant forms thereof, can be expressed via mRNA injection into oocytes; thus, there is no need for generation of stable cell lines. This is a low throughput platform most suitable for confirming hits or lead optimization.

Assay principle

Oocyte impaled with voltage and current electrodes to control and measure the current passing through the channels expressed on the cell membrane.





Example of NMDA receptor subtype 2D

The graph shows the concentration-dependent potentiation of channel activity by incubation with a positive allosteric modulator (PAM) simultaneously with 10 µM each of the co-agonists glutamate and glycine that are necessary for receptor activation.

G-Protein-Coupled Receptor (GPCR) Assays

GPCRs represent the largest individual family of targets for currently approved medications. Recent advances in GPCR pharmacology, including biased signaling and allosteric modulators, have become increasingly important tools in drug discovery. Reaction Biology offers services to progress drug discovery research in the area of GPCR biology and pharmacology.

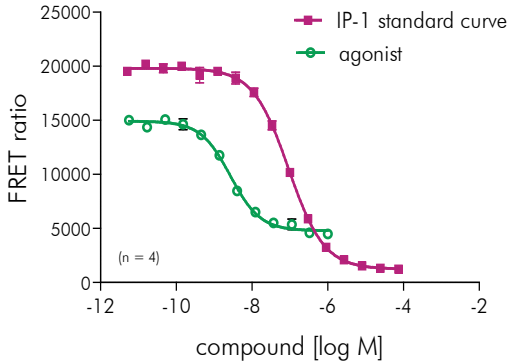
- We offer assay development, high-throughput screening, SAR support services.
- Transmembrane signaling assay formats can be readily established for your receptor of interest including calcium mobilization, ß-arrestin translocation, cAMP generation and inositol 1-monophosphate (IP1) generation

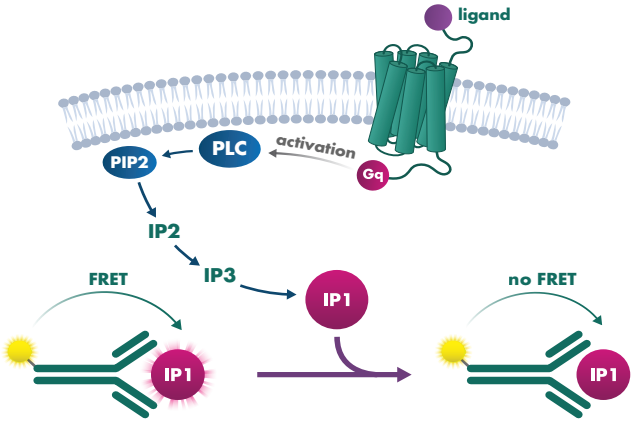
Our dedicated team of GPCR experts will enable drug screening with assays tailored to your specific needs:

1. Define the needs and scope for the project together with our assay development team.
2. Make us familiar with the goals for your research project and define timelines to ensure goal-oriented work right from the start.
3. We will acquire or generate a cell line appropriate to the project needs.
4. The same high standards we use for our off-the-shelf assays will apply to newly developed assays for your project.
5. We guarantee fast turn-around times for data generation.
6. During every step of the process, you will be in close contact with your project manager for regular updates on the study progress.

Agonist-induced IP1 generation

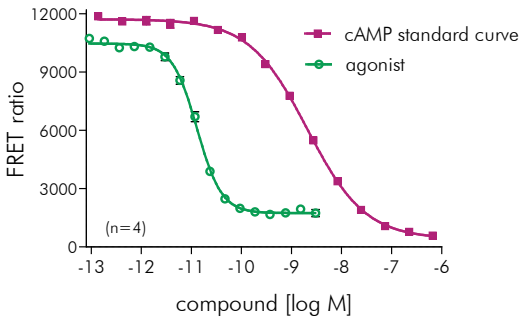
Cells engineered to express Gq-coupled GPCRs of interest are stimulated with an agonist for IP1 accumulation. The generated IP1 is measured using an competitive immunoassay wherein cellular IP1 competes with a labeled IP1 for binding to an anti-IP1-cryptate generating a FRET signal.

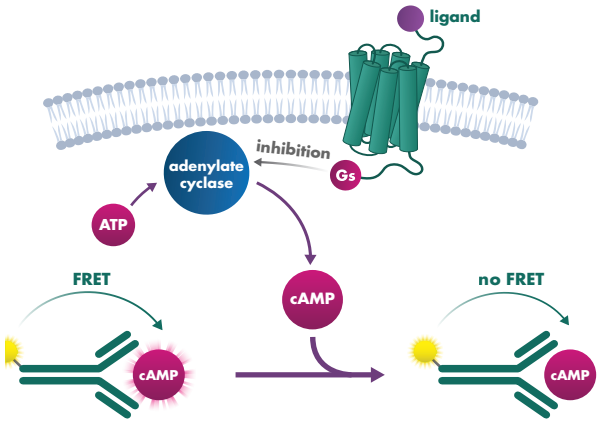




Agonist-induced cAMP generation

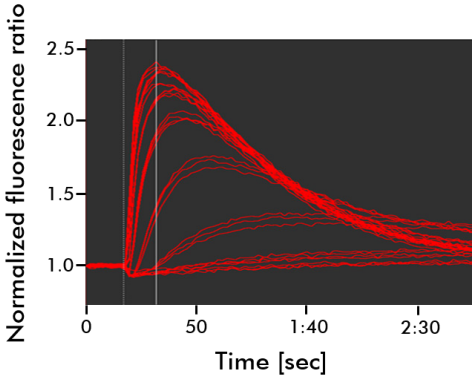
Cells engineered to express Gs-coupled GPCRs of interest are stimulated with an agonist to affect cellular activation. cAMP can be accurately measured by a variety of standard detection methods including a competitive immunoassay wherein cellular cAMP competes with a labeled cAMP probe to bind to an anti-cAMP-cryptate generating a FRET signal.

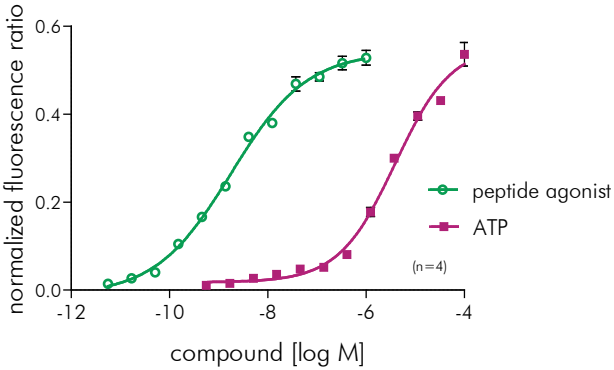




FDSS-based calcium mobilization

CHO cells engineered to express the receptor of interest are loaded with the calcium-sensitive dye Fluo-8 AM. Test compounds are added and fluorescence changes in the cells are monitored over time (left figure). The concentration-response of agonist stimulation for calcium mobilization is shown on the right.

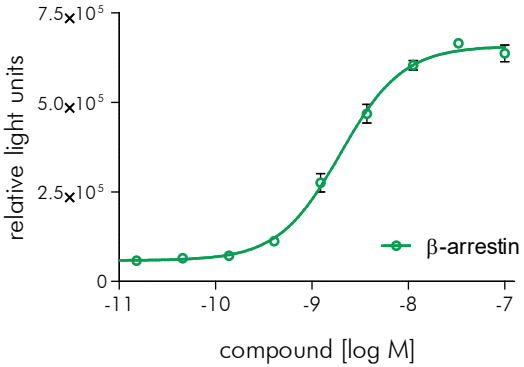




Agonist-induced ß-arrestin translocation

ß-Arrestins bind to activated GPCRs to mediate desensitization and internalization of GPCRs. They are scaffolding proteins that further mediate cell signaling pathways independent of G-proteins.

Example of ß-arrestin translocation in response to treatment with agonist (undisclosed)



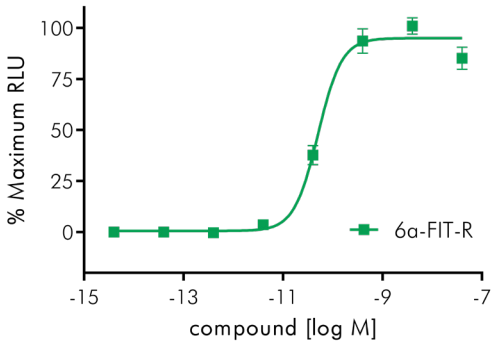
Nuclear Receptor Assays

Nuclear receptors are transcription factors that are regulated by small hydrophobic ligands such as hormones or vitamins. Upon ligand binding, nuclear receptors translocate into the nucleus to bind DNA and modulate the expression of their target genes to regulate a variety of cellular mechanisms such as growth, proliferation, metabolism, or homeostasis on a transcriptional level.

Reaction Biology offers a suite of assays to support the discovery of new drugs to modulate nuclear receptors with the following advantages:

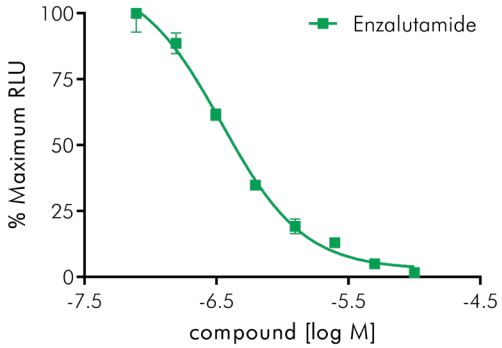
- Cell-based assays allow for drug testing in the physiological and complex environment of intact cells
- Any class of inhibitor can be tested such as modulators of translocation, activity, structural changes, dimerization, etc.
- Custom-assay development

Nuclear Receptors
Androgen receptor (AR)
Farnesoid X receptor (FXR)
Pregnane X receptor (PXR)



AR Agonist Assay:

The androgen receptor reporter cell line was stimulated agonist 6a-FIT-R in various concentrations for 24 hours before luminescence measurements were taken.



AR Antagonist Assay:

The androgen receptor reporter cell line was stimulated with a fixed concentration of agonist 6a-FIT-R and various concentrations of antagonist Enzalutamide for 24 hours before luminescence readout.

Additional Assays

Heat shock protein 90 – HSP90

HSP90 is an ATP-dependent molecular chaperone that stabilizes several regulatory molecules including many tyrosine kinases and transcription factors. Reaction Biology provides compound screening for both HSP90a and HSP90b isoforms.

HSP90
HSP90a
HSP90b

Cytochrome P450 - CYP

CYPs are hemeproteins that play key roles in the metabolism of drugs. Understanding a compound's inhibitory activity against key CYP proteins is essential for predicting drug-drug interactions. Reaction Biology provides compound profiling against the 12 most important CYP isoforms that affect drugs pharmacokinetics and responses.

CYP	
CYP 1A2	CYP 2C19
CYP 19A	CYP 2D6
CYP 2A6	CYP 2E1
CYP 2B6	CYP 2J2
CYP 2C8	CYP 3A4
CYP 2C9	CYP 3A5

Customized Assay Development

Reaction Biology provides protein production and assay development based on customer requests. Targets include enzymes, protein-protein interaction, GPCRs, nuclear receptors, ion channels, and more.

Examples of assays developed for customers

Aldo-keto reductase
Dihydroorotate dehydrogenase
DNA cytidine deaminase
Ectonucleotide pyrophosphatase
GABA aminotransferase
RNAse H2
Transcription factors
RNA polymerase
RNA epigenetic enzymes
PP2A/CIP2A
RASGRP
Sentrin-specific proteases
USP

Assay formats available for customized assay development

AlphaLisa
AlphaScreen
ELISA
Thermal shift assay
Surface plasmon resonance
Microscale thermophoresis
Isothermal titration calorimetry
Flow cytometry
MSD
Electrophysiology
Radiometric assays using ^{3}H, ^{32}P or ^{33}P
Autoradiogram after SDS-PAGE
HTFR
ADP-Glo
Fluorescent peptide screening
NanoBRET
Fluorescence Polarization
FRET
IncuCyte

We have developed over 1,500 assays. Use our vast experience to develop an assay for the target of your interest.

Let’s discover together.

Let's Discover Together.

Recombinant Proteins

- Kinase proteins
- Epigenetic proteins
- Substrates
- Custom-tailored protein production

Target-Specific Assays

- Biochemical and cell-based assays
- Enzymatic activity testing
- Protein: Protein Interaction assays
- Receptor Biology

Cellular Oncology

- 2D and 3D proliferation assays
- Drug combination screening
- Invasion and migration assays
- Angiogenesis assay

Biophysical Assays

- Surface Plasmon Resonance
- Thermal Shift Assay
- Isothermal Titration Calorimetry
- Microscale Thermophoresis

In Vivo Pharmacology

- In Vivo Hollow Fiber Model
- Xenograft models
- Orthotopic models
- Metastasis models

Safety & Adme-Tox

- Cardiac Safety Panel
- CYP inhibition
- PK/PD studies
- In Vitro Safety Panel

Integrated Drug Discovery

- Target research
- Hit identification
- Hit-to-Lead
- Lead optimization

Biomarker Discovery

- Genomic biomarkers
- Protein biomarkers
- Immunophenotyping

Immuno-Oncology

- In Vitro Killing Assays
- Syngeneic Mouse Models
- Propriatary Tumor Models
- Immunophenotyping

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