Promega

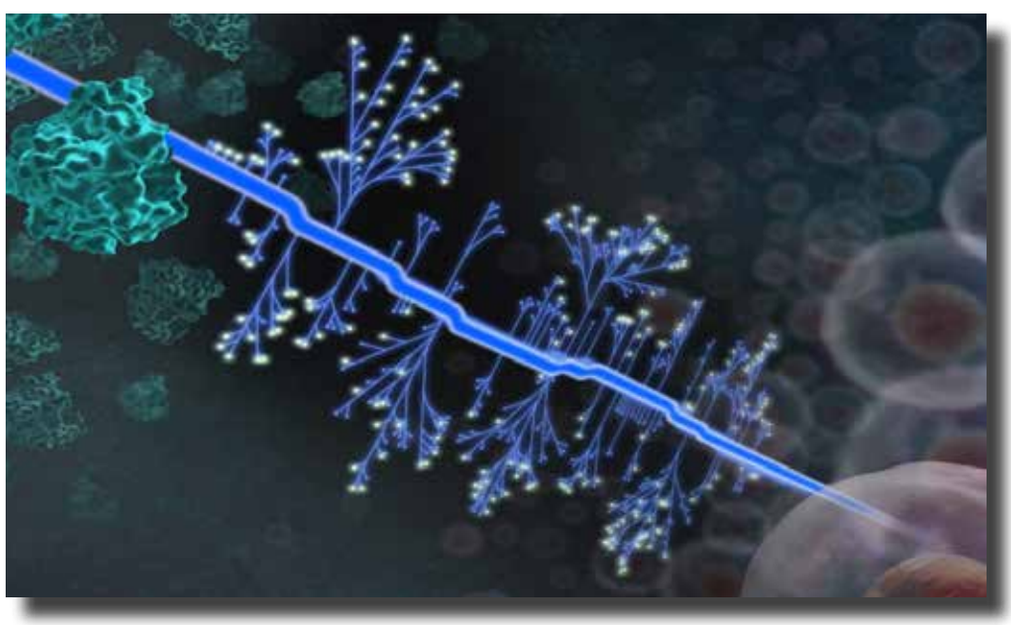
Technologies to Study Kinases

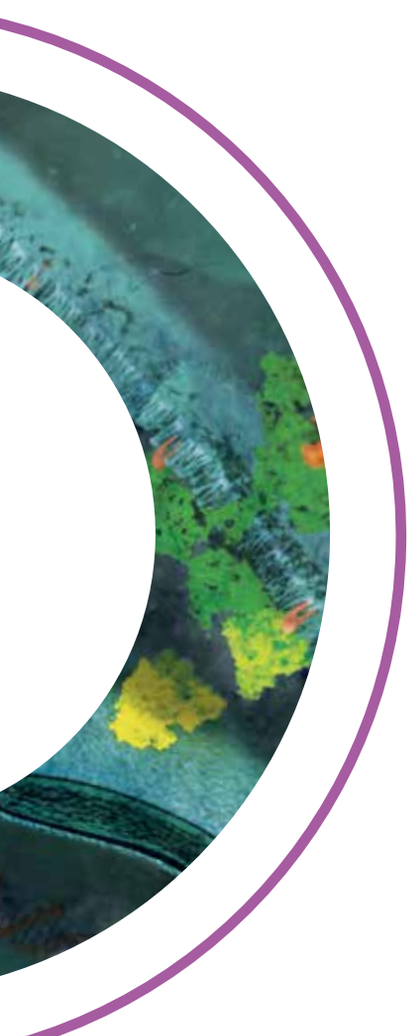
Kinase biology

Kinases are enzymes capable of carrying out phosphorylation events, in which a phosphate group is transferred from ATP to specific sites on lipids or proteins. Protein kinases phosphorylate serine, threonine or tyrosine residues on proteins. Kinases function primarily as components of signalling pathways, in which signals perceived at the cell surface are transduced through the cell by a series of phosphorylation events that ultimately bring about a cellular response. These events modulate the activity of a vast number of proteins, including ion channels, transcription factors, phosphatases and other kinases.

Phosphorylation plays a critical role in signalling pathways that regulate a variety of cellular functions including cell growth, development, differentiation, membrane transport and cell death. Abnormalities in signalling pathways can lead to various pathological conditions including many forms of cancer. For this reason, protein kinases are important targets for both basic research and drug development. There are over 500 protein kinases in the human kinome.

Finding the best tool for measuring kinase activity can be a challenging task and will depend on sample type, available instrumentation and desired throughput. Promega offers a variety of kinase assay systems to measure both kinase activity and compound binding, which include add-mix-measure formats suitable for high-throughput and assays that can be used with virtually any kinase/substrate combination. Tools to study lipid kinases are also available. Here we provide a guide for choosing the best assay for your research needs.





Technologies to study kinases

NanoBRET™ Target Engagement Intracellular Kinase Assay

Measure compound binding at select kinase targets in intact cells. Assess ligand affinity, selectivity, permeability and residence time. The assay is based on the NanoBRET™ System, an energy transfer technique designed to measure molecular proximity in living cells.

Kinase-Glo® Luminescent Kinase Assays

Monitor activity of purified kinases by quantifying ATP. Addition of a single reagent directly to a completed kinase reaction results in the generation of a luminescent signal that is correlated with the amount of ATP present and is inversely proportional to the amount of kinase activity.

ADP-Glo™ Kinase Assay

Measure ADP formed from a kinase reaction. The ADP is converted into ATP, which is used to generate light in a luciferase reaction. The luminescence generated correlates with kinase activity. Well suited for measuring the effects of chemical compounds on the activity of many purified kinases, making it ideal for primary screening as well as kinase selectivity profiling.

Kinase Selectivity Profiling Systems

Profile lead-compound kinase inhibitors against a broad panel of kinases to better understand inhibitor activity and eliminate any off-target effects. Each System includes kinase and substrate pairs organised in an easy-to-use 8-tube strip format optimised for fast and simple kinase profiling reactions. Use with ADP-Glo™ Kinase Assay for bioluminescent detection of kinase activity.

Kinase Enzyme Systems

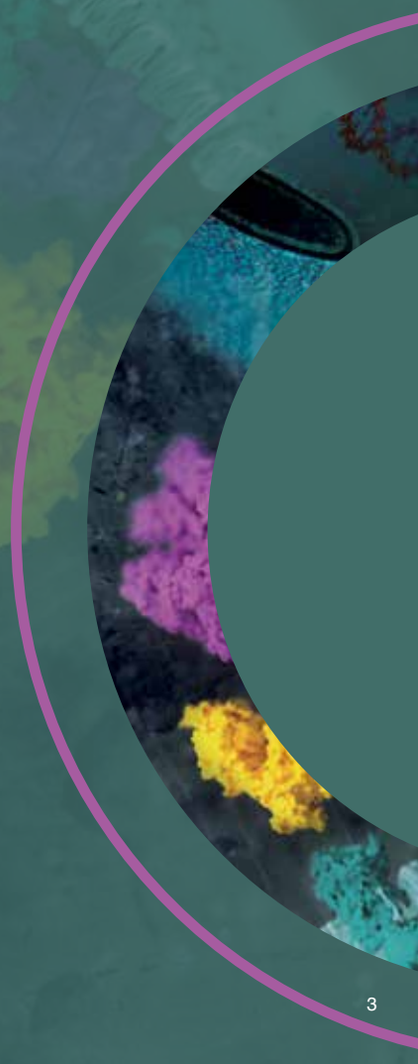
Easily screen and profile inhibitors of a particular kinase. The Systems include a recombinant kinase enzyme, a substrate appropriate for the enzyme, a reaction buffer and supplemental reagents as needed. Use with ADP-Glo™ Kinase Assay for bioluminescent detection of kinase activity.

Lipid Kinase Assay reagents

Substrate and assay reagents for detecting class I phosphoinositide 3-kinase (PI3K) activity. Use in screening assays with purified PI3K class I lipid kinases.

GloMax® Multimode Plate Readers

High performance, simple-to-use multimode readers, integrated with Promega assays to quickly and easily monitor kinase activity and target engagement.



NanoBRET™ TE Intracellular Kinase Assay

Quantitatively measure compound binding at kinase targets in intact cells

Analyse target engagement and binding affinity

NanoBRET Target Engagement (TE) Intracellular Kinase Assay quantitatively measures compound binding at select target kinases in intact cells, in real time. The assay is based on the NanoBRET™ System, an energy transfer technique designed to measure molecular proximity in living cells.

The NanoBRET™ TE Kinase Assay uses four key components:

1) An expressed cellular target kinase fused to the bright NanoLuc® luciferase - these NanoLuc-Kinase Fusion Vectors are available separately;

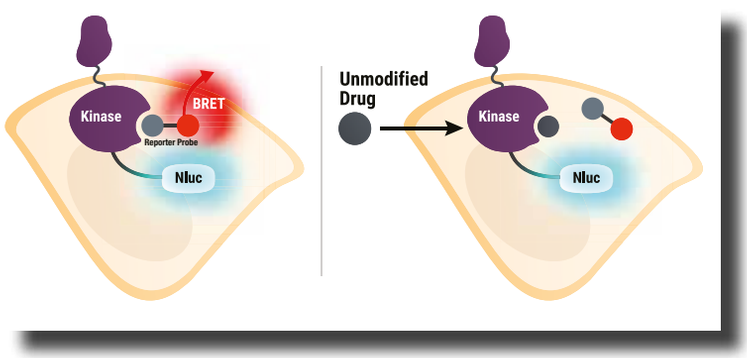
2) A cell-permeable fluorescent tracer - a fluorescently tagged pharmacological probe (K-4 or K-5) that specifically and reversibly binds to the target kinase;

3) A substrate for NanoLuc luciferase; and

4) A cell-impermeable inhibitor for NanoLuc luciferase.

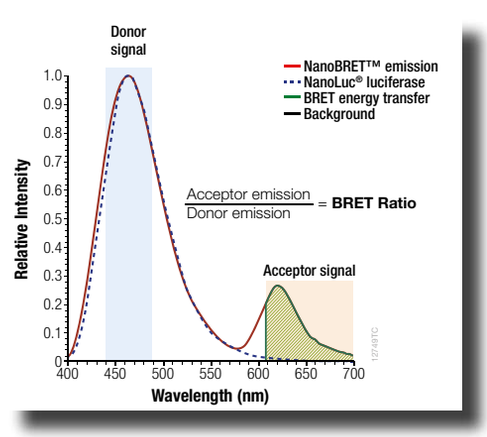
Bioluminescence resonance energy transfer (BRET) is achieved by transferring luminescent energy from the NanoLuc-kinase fusion (BRET donor) to the bound fluorescent tracer (BRET acceptor). Energy transfer is moderated by the proximity of the two partners.

The NanoBRET TE Intracellular Kinase Assay measures the apparent affinity of test compounds for the kinase target by competitive displacement of the NanoBRET tracer. A fixed concentration of tracer (K-4 or K-5) is added to cells expressing the desired NanoLuc-kinase fusion to generate a BRET signal, and the introduction of competing compounds results in a dose-dependent decrease in signal, which allows direct measurement of the permeability and intracellular affinity of the test compound for the target kinase.



In the NanoBRET™ TE Intracellular Kinase Assay, compound engagement is measured in a competitive format using a cell-permeable NanoBRET™ tracer in intact cells. Binding of the test compound results in a loss of NanoBRET™ signal between the target protein and the tracer reversibly bound to the kinase active site.

NanoBRET TE assays have been optimised to use a blue-shifted NanoLuc donor and a red-shifted fluorescent tracer acceptor that have minimal spectral overlap within the assay. This results in optimised signal-to-background ratio and hence an optimised NanoBRET ratio.



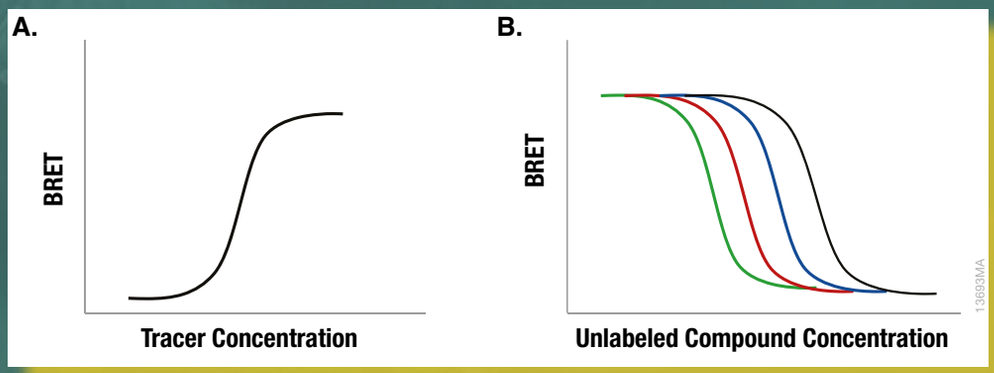
Spectral separation of the NanoLuc emission (460nm) and fluorescent tracer emission (618nm), and calculation of the NanoBRET ratio.

Simple, ready-to-use assay performed in live cells

Measure kinase target affinity and selectivity

Assay uses full-length proteins that may function in complexes within the cell

Traditional biochemical techniques to measure kinase-compound binding or enzymatic inhibition may fail to predict kinase engagement in live cells, as they do not measure compound permeability or take into account the full complexity of the cellular environment that affects target behaviour. The NanoBRET TE Intracellular Kinase Assay allows for the quantitative measurement of compound binding to a diverse set of full-length protein kinases expressed inside living cells, in the presence of cellular factors that are known to impact target engagement potency. The assay performs well at low target-NanoLuc expression levels (similar to endogenous expression).



(A) The optimal NanoBRET™ tracer affinity is determined for each target protein. For analysis of target engagement by a test compound, cells are treated with a fixed concentration of NanoBRET™ tracer that is near the EC_{50} value of the NanoBRET™ tracer dose response curve. (B) To determine test compound affinity, cells are titrated with varying concentrations of the test compound in the presence of a fixed concentration (EC_{50}–EC_{80}) of tracer.

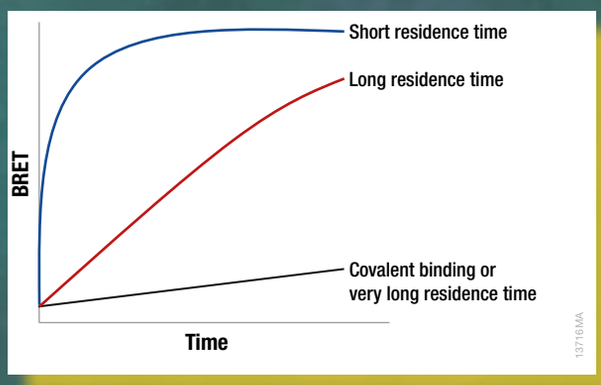
Assays are reproducible and suitable for hit-to-lead optimisation studies

To ensure accurate assessment of intracellular target engagement, a NanoLuc inhibitor is used to mitigate any extracellular NanoLuc signal that may arise from cells compromised during handling, while not adversely affecting NanoLuc luciferase expressed within healthy living cells.

Monitor in-cell residence time

The NanoBRET TE Intracellular Kinase Assay directly measures the durability of compound-target engagement after binding in real time and provides information on full-length protein targets.

Residence time analysis is determined by kinetic analysis of compound dissociation by tracer competition. Cells are incubated with a near-saturating dose of test compound (e.g. IC_{80}), which is allowed to bind to the kinase-NanoLuc fusion. Unbound test compound is removed followed by addition of NanoBRET tracer and detection reagents. Kinetic measurements of the NanoBRET signal are made in order to assess compound residence time.



Addition of compounds with a short residence time will result in a rapid increase in BRET signal upon introduction of the fluorescent tracer. Compounds which bind covalently/ irreversibly or with a long residence time will exhibit a slow/gradual increase in BRET signal over time upon tracer addition.

Choose from >120 kinase fusion vectors

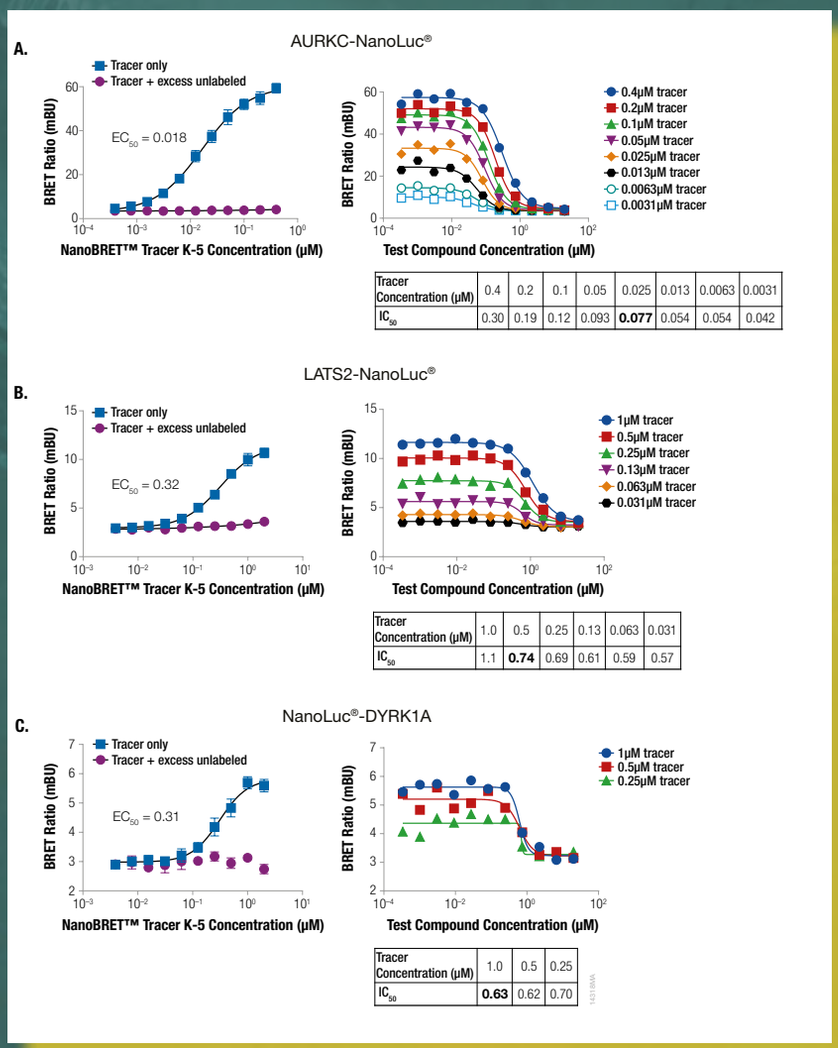
The table below lists all kinase-NanoLuc fusion vectors currently available, each optimised for use with the NanoBRET TE Intracellular Kinase Assays. Each kinase fusion vector has a designated fluorescent tracer, either K-4 or K-5, and this will dictate which assay is needed. The vectors contain either N-terminal or C-terminal fusions of the kinase of interest and NanoLuc luciferase.

For more information visit:
https://www.promega.co.uk/resources/product-guides-and-selectors/kinase-vector-data-selector/

AAK1	CK2α2	ERN1	JNK2	MARK2	PRKAA2	STK16
ABL1	CSF1R	FAK	JNK3	MARK4	PRKACA	STK32B
ALK4	CSK	FER	KIT	MELK	PRKX	STK33
AKT2	DDR1	FGFR1	LATS1	MET	PYK2	STK38
Aurora A	DDR2	FGFR2	LATS2	MLK1	RET	TBK1
Aurora B	DYRK1B	FGFR3	LCK	MUSK	RIOK2	TEC
Aurora C	EPHA1	FGFR4	LIMK2	MYLK2	RIPK2	TEK
AXL	EPHA2	FGR	LTK	NEK2	RSK1	TESK1
BMP2K	EPHA4	FLT3	LYN	NEK3	RSK2	TIE1
BMX	EPHA5	FRK	MAP3K4	NEK9	RSK3	TNK1
BRK	EPHA6	FYN	MAP3K10	NUAK1	RSK4	TRKA
BRSK2	EPHA7	GAK	MAP3K11	P38α	RPS6KA4	TRKB
BTK	EPHA8	IKBKE	MAP3K12	P38β	SIK1	TTK
CDK5	EPHB2	IRAK3	MAP4K1	PAK4	SIK2	TXK
CLK1	EPHB3	IRAK4	MAP4K2	PAK7	SIK3	ULK1
CLK2	EPHB4	ITK	MAP4K3	PHKG1	SLK	ULK2
CLK4	ERK1	JAK3	MAPK4	PKMYT1	SRC	WEE1
CK1γ2	ERK2	JNK1	MAPK6	PLK4	STK11	YES1

In addition to the kinase-NanoLuc fusions listed, many more are available as custom assay materials from Promega Custom Assay Services (CAS) group or are currently in development. Furthermore, Promega can supply a NanoBRET optimised fluorophore dye in ester form for amine-coupling to other pharmacologically active molecules for development of novel tracer molecules for target engagement studies.

The NanoBRET TE Intracellular Kinase Assay is compatible with a diverse set of intracellular kinases and receptor tyrosine kinases (RTKs). Representative target engagement data for three different kinases with different assay windows is shown below. Assay window is defined as the raw fold change in the BRET ratio observed at the recommended concentration of tracer compared to the BRET ratio in the presence of a saturating dose of unlabelled test compound.



Tracer Concentration (µM)	0.4	0.2	0.1	0.05	0.025	0.013	0.0063	0.0031
IC_{50}	0.30	0.19	0.12	0.093	**0.077**	0.054	0.054	0.042

Tracer Concentration (µM)	1.0	0.5	0.25	0.13	0.063	0.031
IC_{50}	1.1	**0.74**	0.69	0.61	0.59	0.57

Tracer Concentration (µM)	1.0	0.5	0.25
IC_{50}	**0.63**	0.62	0.70

1431.BMA

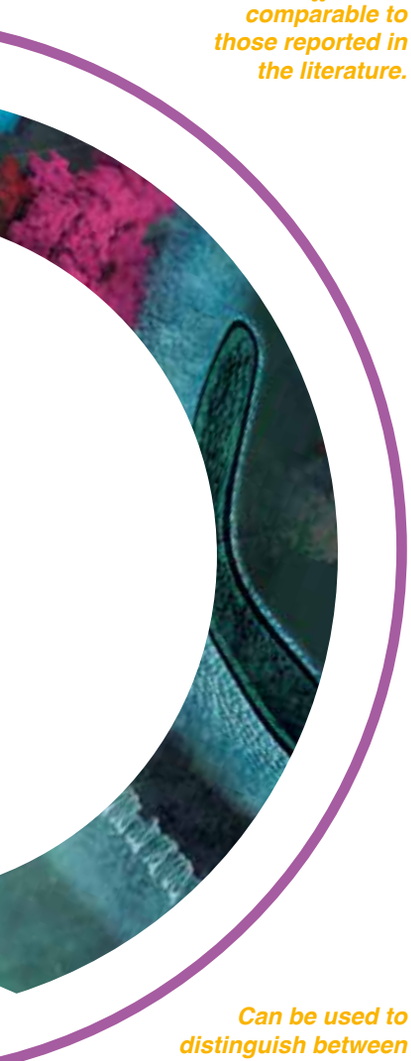
NanoBRET™ tracer K-5 affinity and competition in HEK293 cells transiently expressing NanoLuc®-Kinase fusion proteins. Examples of (A) High Window, (B) Medium Window and (C) Low Window assays are provided. HEK293 cells expressing individual NanoLuc®-Kinase fusions were treated with various concentrations of NanoBRET™ tracer and unlabelled test compound as a competitive inhibitor. BRET was measured after 2 hours using a GloMax® Discover System. BRET ratios were plotted vs. tracer concentration to determine apparent intracellular affinity (EC_{50}) of the tracer. Competitive displacement of tracer by unlabeled test compound was conducted at different fixed concentrations of tracer by plotting BRET ratio vs. test compound concentration. IC_{50} values determined in the presence of the the recommended concentration of tracer for each target are shown in bold.

Ordering information

Product	Size	Cat.No	List Price
NanoBRET™ TE Intracellular Kinase Assay, K-4	100 assays	N2520	£479
NanoBRET™ TE Intracellular Kinase Assay, K-4	1,000 assays	N2521	£2,578
NanoBRET™ TE Intracellular Kinase Assay, K-4	10,000 assays	N2540	£11,047
NanoBRET™ TE Intracellular Kinase Assay, K-5	100 assays	N2500	£479
NanoBRET™ TE Intracellular Kinase Assay, K-5	1,000 assays	N2501	£2,578
NanoBRET™ TE Intracellular Kinase Assay, K-5	10,000 assays	N2530	£11,047
Kinase Fusion Vector for NanoBRET™ Target Engagement	20µg	Kinase-dependent	£358

Kinase-Glo® Luminescent Kinase Assays

Provides IC_{50} values comparable to those reported in the literature.



Can be used to distinguish between ATP-competitive and non-competitive inhibitors

Monitor activity of purified kinases by quantifying ATP

Kinase-Glo® Luminescent Kinase Assays provide a homogeneous, high-throughput screening (HTS) method for measuring kinase activity by quantitating the amount of ATP remaining in solution following a kinase reaction. The ATP is used as a substrate in a luciferase reaction; the luminescent signal is correlated with the amount of ATP present and is inversely correlated with the amount of kinase activity.

Simple

The Kinase-Glo assay is a rapid, 1-step homogeneous assay. A single reagent is added directly to a completed kinase reaction to produce a luminescent signal.

Scalable, reliable and automation-friendly

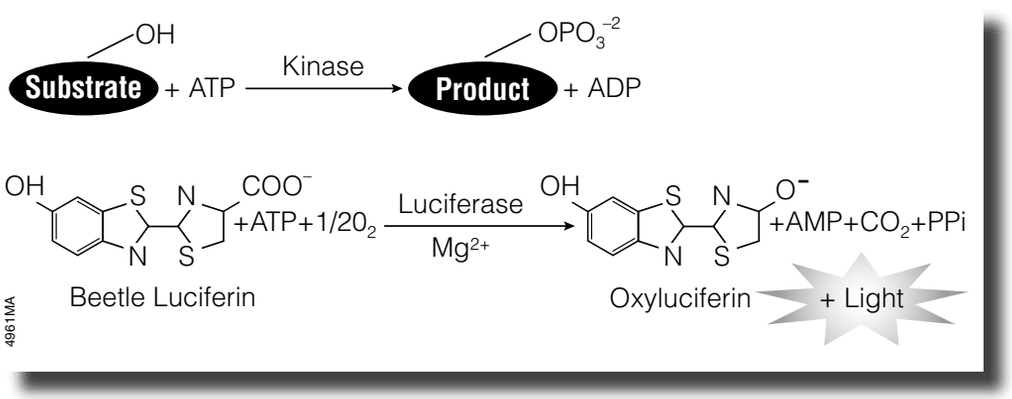
Kinase-Glo Assays are designed for use with multiwell plate formats, making them ideal for automated HTS. The assays produce excellent Z´-factor values of greater than 0.7 in 96- and 384-well formats and easily detect known kinase inhibitors.

Stable luminescent signal

The luciferase reaction uses a proprietary thermostable luciferase (Ultra-Glo™ Recombinant Luciferase), formulated to generate a stable 'glow-type' luminescent signal that has a half-life of greater than 5 hours, allowing batch plate processing.

Use any kinase and kinase-substrate combination

These assays can be performed with virtually any kinase and substrate combination, and without substrate modifications. Substrates include peptides, proteins, lipids and sugars.

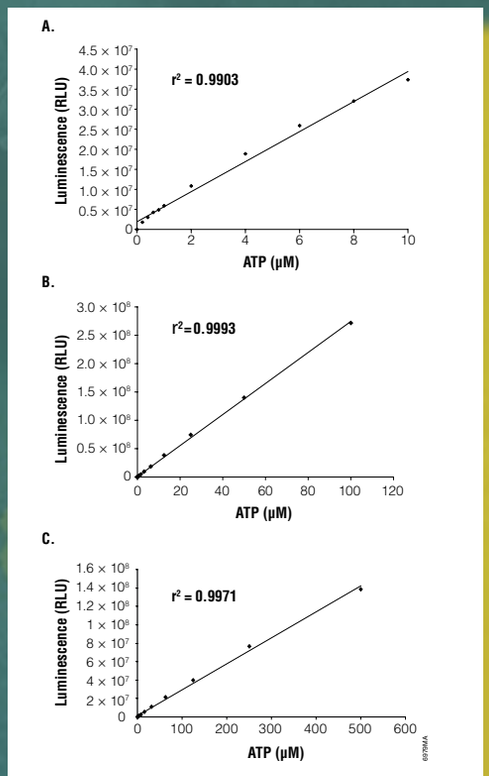


The Kinase-Glo® Assay reaction. ATP remaining following a kinase reaction at the time that the reagent is added is used as a substrate by the Ultra-Glo™ Luciferase to catalyse the mono-oxygenation of luciferin. Luminescence is inversely related to kinase activity.

Choose your assay format

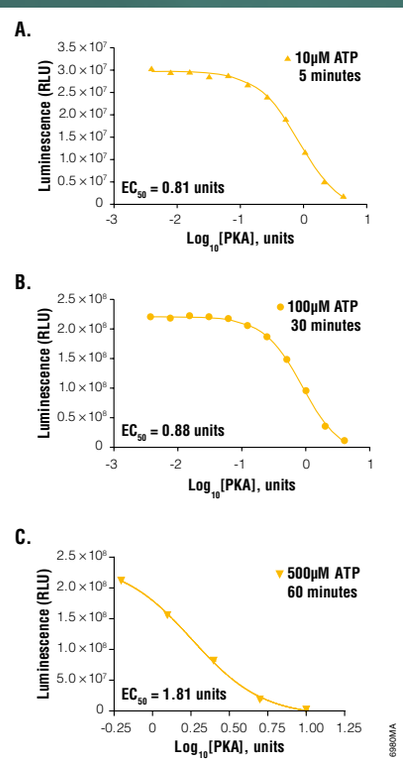
The Kinase-Glo® Platform consists of three assay formats, differentiated by their linear response to ATP: the Kinase-Glo® Assay, which is used to monitor kinase activity using up to 10µM ATP; the Kinase-Glo® Plus Assay, which is used for assays requiring higher ATP concentrations (up to 100µM); and the Kinase-Glo® Max Assay, which is used for assays requiring up to 500µM ATP, making it well suited for use with kinases with high Km for ATP as well as for screening for kinase inhibitors that do not compete at the ATP binding site.

Use higher ATP concentrations



Luminescence correlates with amount of ATP. Serial dilutions of ATP were made and an equal volume of the appropriate Kinase-Glo® Reagent was added to each well. Luminescence was recorded using a GloMax® luminometer. There is a linear relationship between the luminescent signal and the amount of ATP in the kinase reaction buffer from (A) 0-10µM using the Kinase-Glo® Assay (B) 0-100µM using the Kinase-Glo® Plus Assay and (C) 0-500µM using the Kinase-Glo® Max Assay.

Three kit formats



An inverse relationship exists between luminescence measured with the Kinase-Glo® Reagent and the amount of kinase activity. Kinase reactions were performed at (A)10µM ATP, 50µM Kemptide substrate (Cat.# V5601) with 1 unit of PKA for 5 minutes using the Kinase-Glo® Assay (B) 100µM ATP, 500µM Kemptide substrate and 1.5 units of PKA for 30 minutes using the Kinase-Glo® Plus Assay or (C) 500µM ATP, 500µM Kemptide and 1.5 units of PKA for 60 minutes using the Kinase-Glo® Max Assay. Following the kinase reaction, an equal volume of Kinase-Glo® Max Reagent was added. Luminescence was recorded on a GloMax® luminometer after ten minutes.

Ordering information

Product	Size	Cat.No	List Price
Kinase-Glo® Assay	10ml	V6711	£62
Kinase-Glo® Assay	10 x 10ml	V6712	£397
Kinase-Glo® Assay	100ml	V6713	£316
Kinase-Glo® Assay	10 x 100ml	V6714	£2,370
Kinase-Glo® Plus Assay	10ml	V3771	£68
Kinase-Glo® Plus Assay	10 x 10ml	V3772	£412
Kinase-Glo® Plus Assay	100ml	V3773	£343
Kinase-Glo® Plus Assay	10 x 100ml	V3774	£2,595
Kinase-Glo® Max Assay	10ml	V6071	£74
Kinase-Glo® Max Assay	10 x 100ml	V6072	£428
Kinase-Glo® Max Assay	100ml	V6073	£378
Kinase-Glo® Max Assay	10 x 100ml	V6074	£2,772

ADP-Glo™ Kinase Assay & ADP-Glo™ Max Assay

An assay for all your kinase screening needs

ADP-Glo Kinase Assay provides a universal, homogeneous, high-throughput screening (HTS) method to measure kinase activity, by quantifying the amount of ADP produced during a kinase reaction. The ADP is converted into ATP, which is used to generate light in a luciferase reaction. The luminescence generated is proportional to the ADP concentration produced and correlates with kinase activity.

The assay is well suited for measuring the effects of chemical compounds on the activity of many purified kinases, making it ideal for primary screening as well as kinase selectivity profiling.

Universal assay

The activity of virtually any ADP-generating enzyme (e.g. kinase or ATPase) using up to 1mM ATP can be monitored using the ADP-Glo Kinase Assay. The assay can be performed with virtually any kinase and substrate combination including peptide, protein, lipid or sugar substrates and does not require radioactively labelled components or antibodies.

The ADP-Glo™ Max Assay should be used when ATP concentrations of up to 5mM are required.

Stable luminescent signal

The luciferase reaction uses a proprietary thermostable luciferase (Ultra-Glo™ Recombinant Luciferase) and the signal produced is stable for more than 3 hours, allowing batch plate processing without the need for strictly timed incubations.

Sensitive to low ADP concentrations

ADP-Glo Kinase Assay is sensitive down to 20nM ADP in 10µl (0.2pmol), meaning less enzyme is required per well. The assay can be used with essentially all kinases due to its high sensitivity.

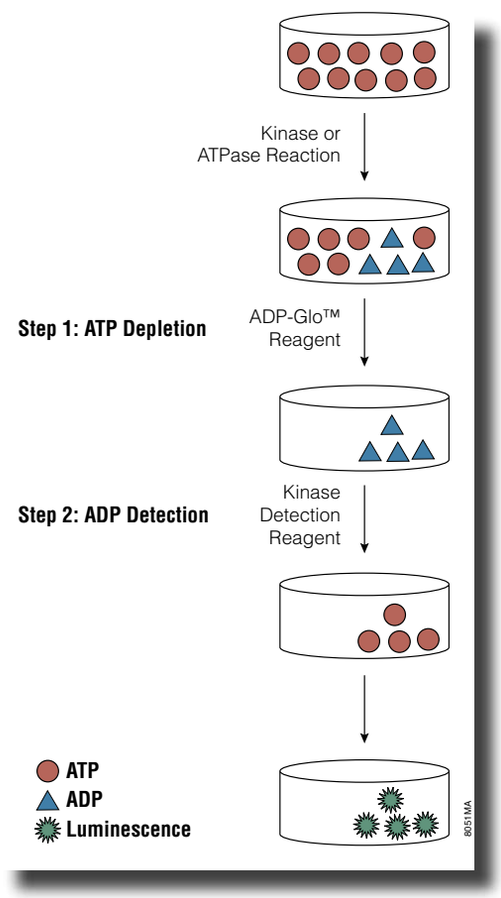
Large dynamic range

High signal-to-background ratios at lower percent conversions of ATP to ADP allow the use of smaller amounts of enzyme, ideal for high-throughput screening.

Broad range of ATP concentrations

The assay is linear from µM to mM range, allowing distinction between ATP-competitive and non-competitive inhibitors.

Principle of the ADP-Glo Kinase Assay

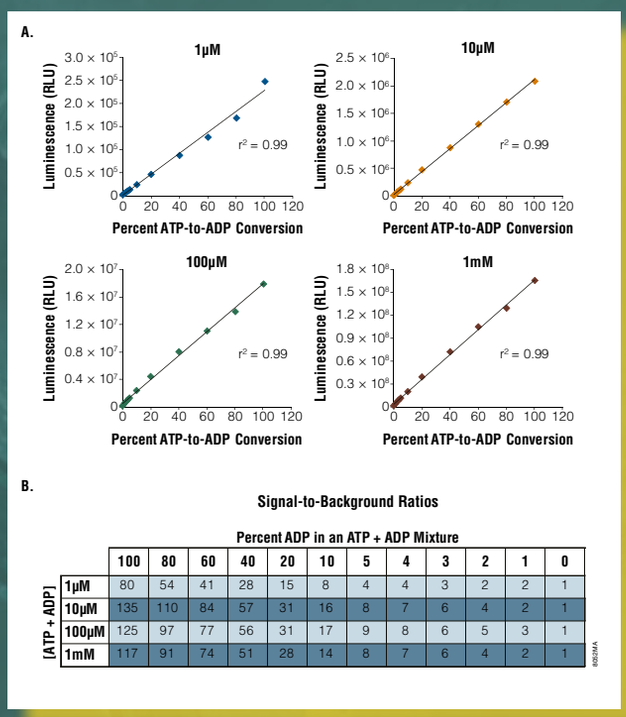


The assay is performed in two steps:
1) ATP depletion: After the kinase or ATPase reaction, ADP-Glo™ Reagent is added to terminate the kinase reaction and deplete the remaining ATP.
2) ATP detection: Kinase Detection Reagent is added to convert ADP to ATP and allow the newly synthesised ATP to be measured using a luciferase/luciferin reaction. The light generated correlates to the amount of ADP generated, which is indicative of kinase or ATPase activity.

Simple 2-step assay protocol

Screen library compounds for effects on target kinases

Sensitivity and linearity of the ADP-Glo Kinase Assay



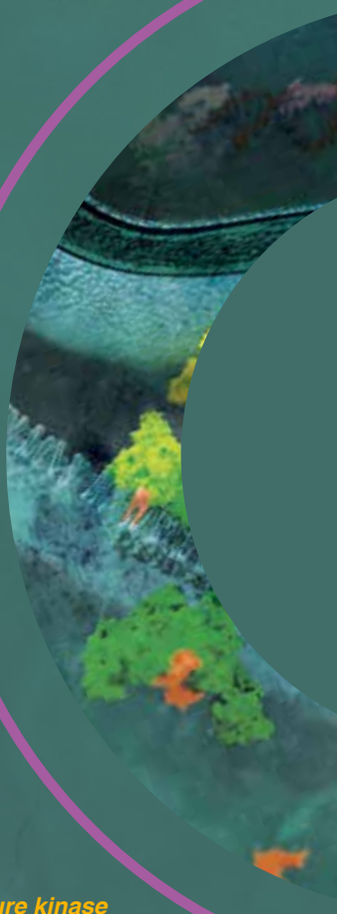
B.

Signal-to-Background Ratios

Percent ADP in an ATP + ADP Mixture

[ATP + ADP]	100	80	60	40	20	10	5	4	3	2	1	0
1µM	80	54	41	28	15	8	4	4	3	2	2	1
10µM	135	110	84	57	31	16	8	7	6	4	2	1
100µM	125	97	77	56	31	17	9	8	6	5	3	1
1mM	117	91	74	51	28	14	8	7	6	4	2	1

(A) Four ATP-to-ADP conversion curves were prepared at the indicated ATP+ADP concentrations and luminescence was recorded using a GloMax® Luminometer. There is a linear relationship between the luminescent signal and the amount of ADP in the reaction buffer at all ATP+ADP concentration series tested. (B) The ADP-Glo™ Kinase Assay is highly sensitive as shown by the high signal-to-background ratios.



ADP-Glo displays high signal strength at low ATP conversion

Enzyme	SB5	% ATP to ADP conversion at SB5
EGFR	0.3ng	2.2
PDGFRa	21ng	4.7
VEGFR2 (KDR)	1ng	0.2
DNA-PK	0.4ng	2.7
AKT2	7ng	4.3
IKKb	4.5ng	2.9
MAPK (ERK2)	1ng	5.6
PKA	0.0123 units	0.35
Hexokinase	0.1units	4.6
PI3 kinase g	0.15ng	2.0
Sphingosine kinase 1	0.8ng	2.9

ADP-Glo™ Kinase Assay produces high signal-to-background ratios (SB) with all kinase enzymes tested. Generating a signal-to-background ratio of 5 (SB5) requires only small amounts of enzyme per reaction (indicated in the column labelled SB5). To generate an SB5 the percentage of ATP converted to ADP is 0.2-6% for the enzymes listed.

Measure kinase activity that more closely mimics physiological conditions

Z'-Factors >0.7 are routinely obtained

Ordering information

Product	Size	Cat.No	List Price
ADP-Glo™ Kinase Assay	400 assays	V6930	£155
ADP-Glo™ Kinase Assay	1,000 assays	V9101	£359
ADP-Glo™ Kinase Assay	10,000 assays	V9102	£2,034
ADP-Glo™ Kinase Assay	10 x 10,000 assays	V9103	£14,070
ADP-Glo™ Kinase Assay, Bulk Packaged	100,000 assays	V9104	£13,529
ADP-Glo™ Max Assay	1,000 assays	V7001	£359
ADP-Glo™ Max Assay	10,000 assays	V7002	£2,034

Kinase Selectivity Profiling Systems

Profile compounds in-house with pre-optimised kinase assays

Kinase Selectivity Profiling Systems are ideal for profiling lead-compound kinase inhibitors against a broad panel of kinases during the drug discovery process, to better understand inhibitor activity and to obviate any off-target effects. Each System includes kinase and substrate pairs organised in an easy-to-use 8-tube strip format, optimised for fast and simple kinase profiling reactions. They can also be combined with ADP-Glo™ Kinase Assay technology to measure ADP formed from a kinase reaction.

Assays can be performed manually, or automated on a liquid-handler

Fast turnaround time

Lead compounds can be profiled in-house in hours, not days. Two quick, one-step dilutions provide working stocks of kinases and substrate/cofactor solutions.

Streamlined profiling of hits and leads for potency against kinase families

Kinases from single kinase families are either grouped together for more relevant selectivity profiles, or are available as a general panel of kinases representative of the human kinome, for a broad kinase profile.

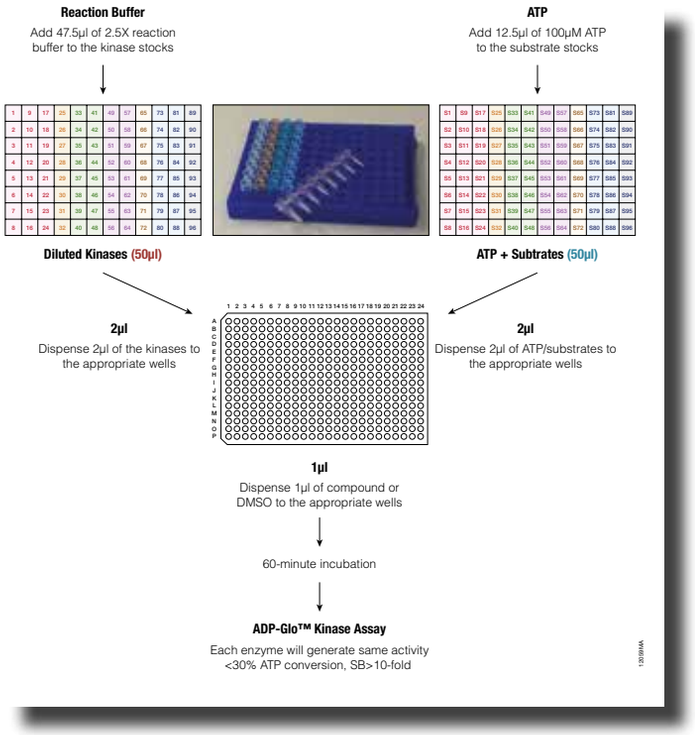
Optimised kinase activity

All kinases have been developed for optimal ADP production, with a signal-to-background ratio of ≥10 when assayed at 10µM ATP using the ADP-Glo Kinase Assay.

Stable luminescent signal

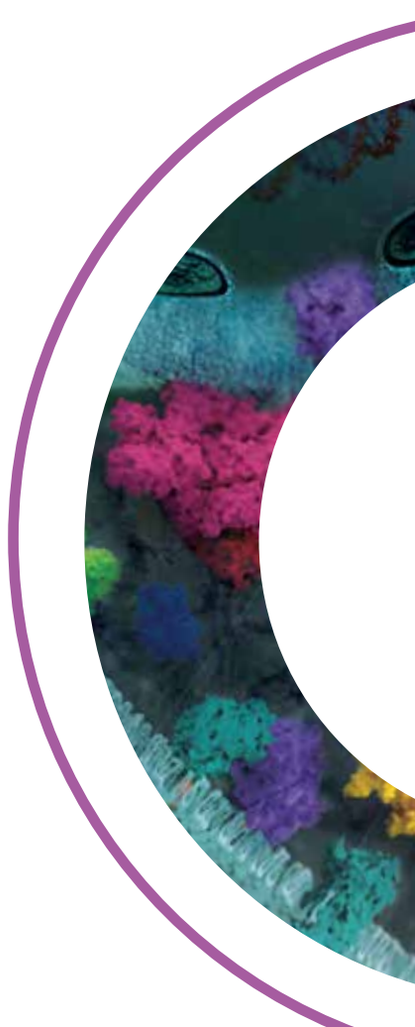
Batch plate processing can be performed without the need for strictly timed incubations, making the process flexible. The Systems are optimised for performance in a 384-well plate and are well suited for high-throughput kinase selectivity profiling and automated setup.

No need to send precious compounds to a third party



Simple, Automation-Friendly Assay, No Time-Consuming Optimisation

Concentrated kinases and substrates/cofactor stocks are first diluted and then combined with test compound in a 384-well plate. After a 1-hour incubation, the ADP-Glo™ Assay is performed. The resulting luminescent signal is proportional to ADP concentration and correlates with kinase activity.



Custom kinase assay strips available

If a different combination of kinases is needed than those provided in our catalogue, you can design your own custom profiling system using our Custom Kinase Profiling System Tool online:

https://www.promega.co.uk/products/cell-signalling/kinase-assays-and-kinase-biology/customize/

Flexible kinase inhibitor profiling

Kinase Selectivity Profiling Systems and optimised protocols can be used to easily generate selectivity profiles using small or large kinase panels, and to detect compound promiscuity towards members of a single kinase subfamily or different subfamilies of the kinome.

Get results faster by profiling lead compounds for kinase selectivity in-house!

Each Kinase Selectivity Profiling System provides enough material to profile up to 20 compounds at a single dose:

Single-Dose Profiling

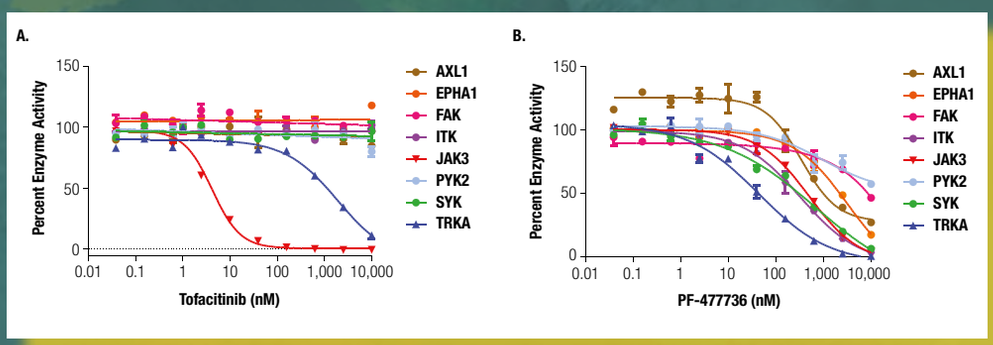
>60% Activity	20–60% Activity	<20% Activity

Kinase Profiling System: TK-3	Gefitinib	Dasatinib	Tofacitinib	SB203580	Roscovitine	PF-477736	Tozasertib	Enzastaurin
AXL1	108	92	105	94	102	54	107	101
EPHA1	84	1	102	79	97	63	77	100
FAK	95	84	118	106	109	86	91	115
ITK	102	101	99	94	100	33	24	99
JAK3	99	113	0	87	94	36	88	103
PYK2	133	134	122	106	134	120	80	116
SYK	104	72	84	87	94	34	77	103
TRKA	95	82	60	89	87	9	1	89

Inhibitor Concentration = 1µM

Single-dose inhibitor profiles of eight compounds created using the Kinase Selectivity Profiling System: TK-3. Kinase activity was quantified using the ADP-Glo™ Kinase Assay. The % activity of each kinase in the presence of each compound is indicated. Known kinase inhibitors (in red) were easily identified.

Or as a dose-response curve for two compounds against each of the eight kinases in the Kinase Strip:



Inhibitor dose-response curves created using the Kinase Selectivity Profiling System: TK-3. Kinase activity was quantified using the ADP-Glo™ Kinase Assay. Dose response curves are shown for (A) the selective JAK3 inhibitor tofacitinib and (B) non-selective inhibitor PF-477736.

Ordering information

Product	Size	Cat.No	List Price per product
Kinase Selectivity Profiling System: TK1, TK2, TK3, TK4	8 x 50 reactions each	V6850 V6852 V6920 V6922	£386
Kinase Selectivity Profiling System: CAMK1, CAMK2	8 x 50 reactions each	V6932 V6924	£386
Kinase Selectivity Profiling System: AGC-1, AGC-2	8 x 50 reactions each	V6858 V6910	£386
Kinase Selectivity Profiling System: CMGC-1, CMGC-2	8 x 50 reactions each	V6854 V6856	£386
Kinase Selectivity Profiling System: STE,-1, TKL-1	8 x 50 reactions each	V6916 V6914	£386
Kinase Selectivity Profiling System: Other/CK-1, Other-2	8 x 50 reactions each	V6918 V6926	£386
Kinase Selectivity Profiling System: General Panel	8 x 50 reactions each	V6928	£938

Kinase Selectivity Profiling Systems are also available with ADP-Glo™ Kinase Assay reagents.

Kinase Enzyme Systems

Convenient, scalable kinase profiling

Kinase Enzyme Systems allow you to easily screen and profile inhibitors of a particular kinase. These Systems, created in partnership with SignalChem, span the breadth of the human kinome. They provide all of the optimised components (enzyme, preferred substrate, required cofactors and buffer) needed to generate a kinase selectivity profile for a compound. By using this single platform, lead compounds can be easily profiled in-house in a cost-effective manner with no loss in sensitivity.

All Kinase Enzyme Systems have been optimised and quality controlled with the ADP-Glo™ Kinase Assay. The Kinase Enzyme Systems can be purchased with or without the ADP-Glo Kinase Assay reagents. Used together, the ADP-Glo Kinase Assay and Kinase Enzyme Systems provide a convenient method for profiling the effect of lead compounds on kinase activity.

Profile more compounds in-house

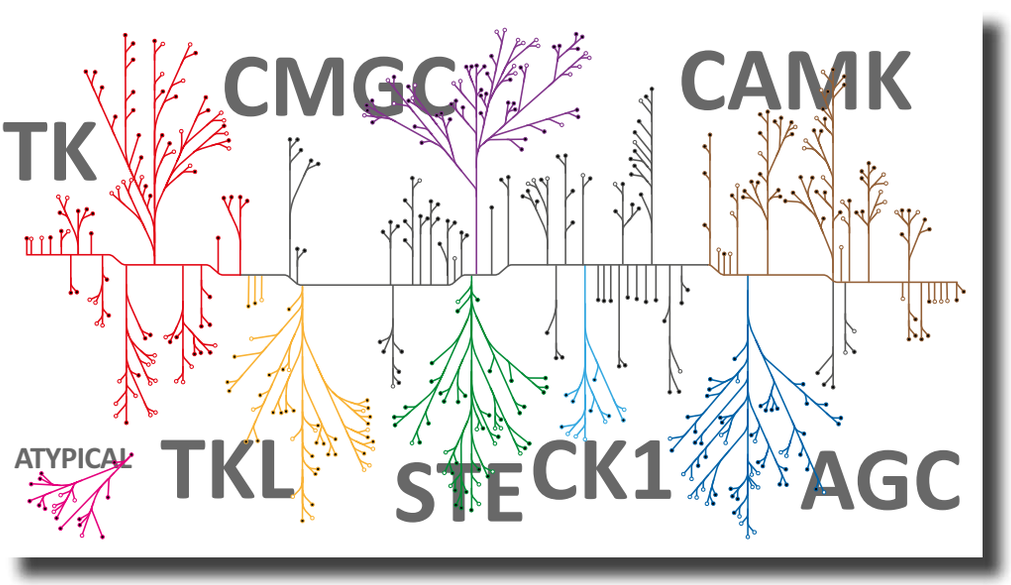
Both the ADP-Glo Kinase Assay and the Kinase Enzyme Systems are optimised, saving time and reagent costs.

Complete solution

The Kinase Enzyme Systems include a recombinant kinase enzyme, a substrate appropriate for the enzyme, a reaction buffer, DTT and supplemental reagents as needed.

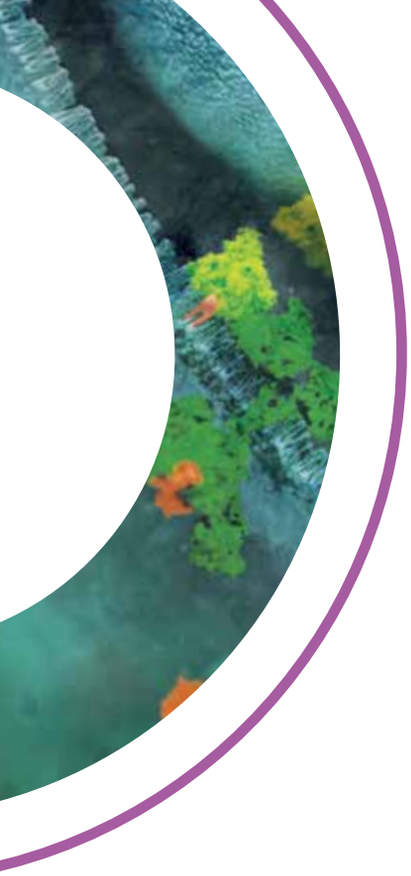
Obtain reliable results

The broad dynamic range, ease of use and better sensitivity obtained with ADP-Glo Kinase Assay result in less ambiguous data.



For more information about Kinase Enzyme Systems, visit: *www.promega.co.uk/KinaseTool*

Kinase Enzyme System	Kinase Enzyme System Cat.#	Kinase Enzyme System	Kinase Enzyme System Cat.#	Kinase Enzyme System Cat.#	Kinase Enzyme System Cat.#
ABL1	V1901	FER	V1981	p38δ	V4078
ABL1 (E255K)	V5098	FGFR1	V2991	p70S6K	V2741
ABL1 (T315I)	V5320	FGFR2	V4060	p70S6Kb	V4030
ABL1 (Y253F)	V5086	FGFR3 (K650E)	V5082	PAK1/CDC42	V4478
ACK	V4050	FGFR4	V4062	PAK3	V4080
AKT1	V1911	FLT1	V3001	PAK4	V3201
AKT2	V3861	FLT3	V4064	PASK	V4240
AKT3	V4010	FLT3 (D835Y)	V4514	PDGFRα	V3721
ALK2	V4492	FMS	V4022	PDGFRα (D842V)	V4480
ALK4	V4508	FYN A	V3571	PDGFRα (T674I)	V4486
AMPK (A1/B1/G1)	V1921	GRK5	V3981	PDGFRβ	V3731
AMPK (A1/B1/G2)	V4012	GSK3α	V3051	PDK1	V2761
AMPK (A2/B1/G1)	V4014	GSK3β	V1991	PIM1	V4032
ASK1	V3881	HER2	V3891	PIM2	V4034
Aurora A	V1931	HER4	V3101	PKA	V4246
Aurora B	V3971	HIPK1	V4066	PKCα	V3381
AXL	V3961	HIPK3	V4164	PKCβI	V5094
BMX	V4512	HPK1	V4098	PKCβ II	V3741
BRK	V4054	IKKα	V4068	PKCγ	V3391
BTK	V2941	IKKβ	V4502	PKCδ	V3401
CAMK1 γ	V4016	IGF1R	V3581	PKCε	V4036
CAMK1 α	V4018	InsR	V3901	PKCζ	V2781
CAMK2γ	V3531	IRAK4	V2621	PKCθ	V4040
CAMK4	V2951	ITK	V3191	PKCι	V3751
CAMKK1	V4470	JAK3	V3701	PKCμ	V4038
CDK1/CyclinA2	V2961	JNK1	V4070	PKD2	V4042
CDK2/CyclinA2	V2971	JNK3	V3821	PLK1	V2841
CDK2/CyclinE1	V4488	KDR	V2681	PYK2	V4082
CDK3/CyclinE1	V4490	KHS1	V4108	RET	V3761
CDK5/p25	V3231	c-KIT	V4498	RET (V804L)	V4472
CDK5/p35	V3271	LCK	V2691	RET (Y791F)	V5326
CDK6/CyclinD3	V4510	LRRK2	V4474	RIPK2	V4084
CDC7/DBF4	V5088	LYN B	V3711	ROCK1	V3411
CDK9/Cyclin K	V4104	MAPKAPK2	V4024	ROCK2	V4044
CHK1	V1941	MAPKAPK3	V4026	RON	V3921
CHK2	V4020	MAPKAPK5	V4166	RSK 1	V4046
CK1 α1	V4484	MARK1	V4028	RSK 2	V3501
CKγ1	V4100	MELK	V4150	SGK1	V2911
CK2 α1	V4482	c-MER	V3541	SIK	V4156
CK1 ε	V4160	MET (M1250T)	V4168	SLK	V4242
CLK1	V4056	MET	V3361	SRC	V2921
CLK3	V4162	MINK1	V3911	STK33	V4086
CSK	V2981	MLCK	V4496	SYK	V3801
DAPK1	V4096	MLK1	V4072	TAK1-TAB1	V4088
DDR2	V4058	MRCK	V5710	TAOK1	V4090
DNA-PK	V4106	MLK2	V4476	TBK1	V3991
DYRK2	V5090	MSK1	V5092	TGFβR1	V4092
EGFR	V3831	MSK2	V5080	TGFβR2	V3931
EGFR (L858R)	V5322	MST1	V4152	TNIK	V4158
EGFR (L861Q)	V4102	MYO3b	V4074	TOPK	V4094
EGFR (T790M)	V4506	NEK2	V3871	TRKA	V2931
EGFR (T790M, L858R)	V5324	NEK3	V4500	TRKB	V4048
EIF2AK2	V5328	NIK	V4076	ULK1	V3521
EPHA1	V3561	NUAK2	V5096	VRK2	V4494
ERK1	V1951	p38α	V2701	WNK1	V5084
ERK2	V1961	p38β	V4154	ZAK	V4244
FAK	V1971	p38γ	V3371	ZAP70	V8311



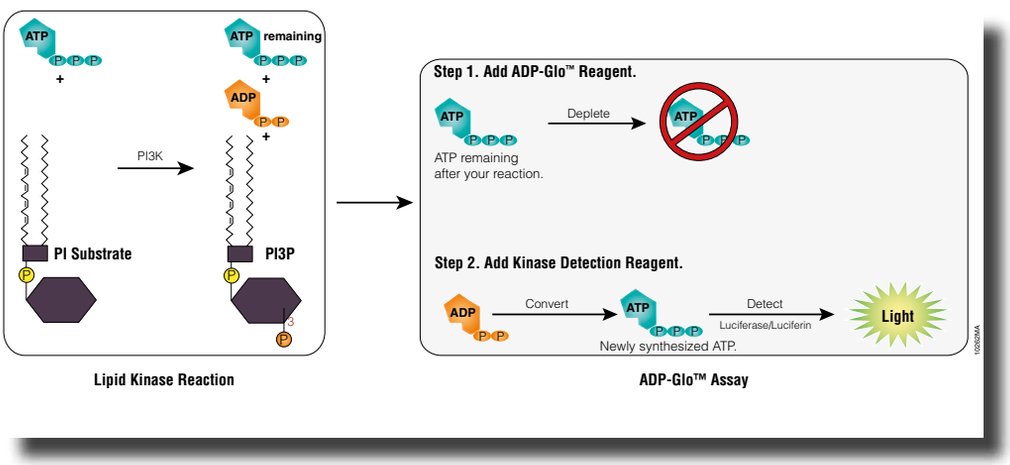
Lipid Kinase Assay Reagents

Complete solution to sensitively detect class I PI3 kinases

For selectivity profiling or high throughput screening, the ease and sensitivity of the ADP-Glo™ Kinase Assay combined with high quality lipid kinases and substrates enables compound profiling at any throughput level.

The complete set of reagents includes purified human recombinant proteins of class I phosphoinositide 3-kinases, optimised reaction buffer and ready-to-use lipid kinase substrates. The PI3K enzymes available include α, ß, γ and δ as well as two mutants of alpha (E545K and H1047).

Procedure for detection of PI3 Kinases with ADP-Glo Assay



Principle of the ADP-Glo™ Kinase Assay featuring PI3Ks. (A) The lipid kinase reaction, (B) ADP-Glo Kinase Assay has 2 steps. Step 1: ADP-Glo Reagent is added to terminate the lipid kinase reaction and deplete the remaining ATP. Step 2: Kinase Detection Reagent is added to convert ADP to ATP. The newly synthesised ATP drives a luminescent reaction with luciferase/luciferin to produce light. The amount of light produced is proportional to the original lipid kinase activity.

Complete solutions for Class I PI3Ks

Purified recombinant enzymes with high specific activity combined with ready-to-use lipid substrates (PI or PIP2) and a universal reaction buffer formulation are provided.

Profile more compounds at any throughput

Universal assay

Virtually any kinase-substrate combination can be used, including peptide, protein, lipid and sugar substrates. A wider range of kinases can be screened in-house, reducing dependency on costly outsourcing.

High signal strength at low ATP conversion

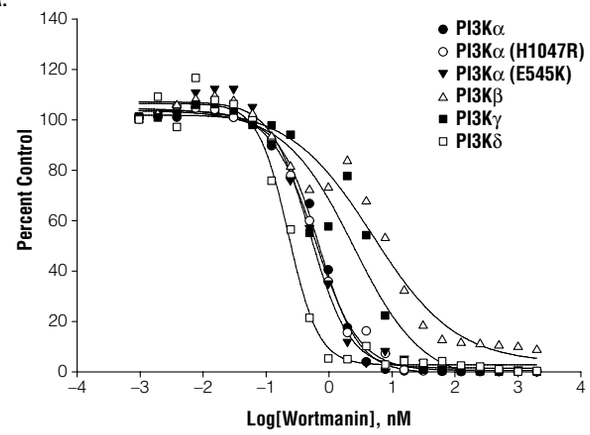
Kinase activity is measured under conditions more closely mimicking physiological conditions, making the assay well suited to low-activity kinases.

Wide range of ATP levels

Assays are linear from µM to mM range, allowing distinction between ATP competitive and non-competitive kinase inhibitors.

PI3 Kinase inhibitor potency determined using ADP-Glo Assay

A.



B.

Isoform	PI3K (nM)	IC_{50} (nM)
α	1.1	0.68
α(E545K)	0.79	0.6
α(H1047R)	0.61	0.5
β	5	4.9
γ	2	2.4
δ	0.8	0.2

10257MA

One assay platform meets the substrate requirement of all kinases

Example of general PI3K inhibitor potency data. (A) IC_{50} values of the known PI3K inhibitor wortmanin were determined using the protocol described in Promega Technical Manual, #TM365. No-enzyme (background) control values were subtracted from all data points, and the percent inhibition was calculated relative to enzyme activities in the absence of inhibitor (100% activity). Data were plotted using the sigmoidal dose-response, variable slope model supplied with SigmaPlot™ 9.0 software. (B) The amount of enzyme used in the reactions and the calculated IC_{50} values for different class I PI3K isoforms.

Ordering information

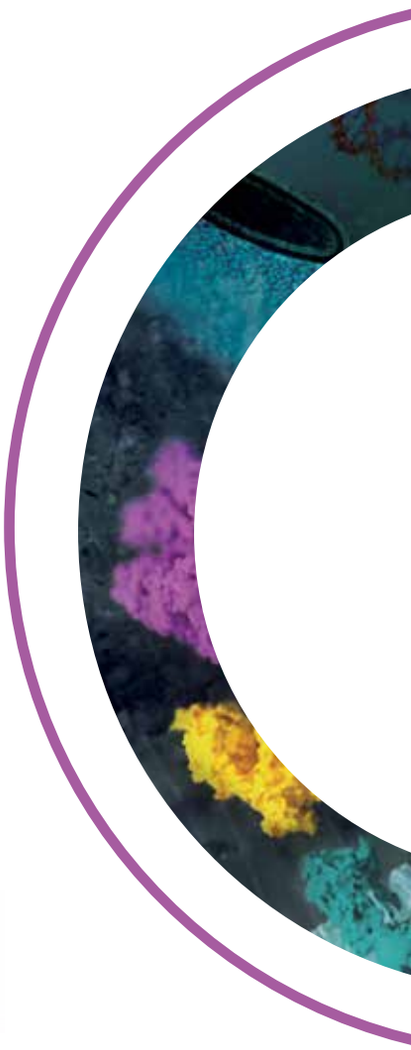
Product	Size	Cat.No	List Price
PI3K-Glo™ Class I Profiling Kit	1 each	V1690	£864
ADP-Glo™ Kinase Assay with PI:3PS	1,000 assays	V1781	£539
ADP-Glo™ Kinase Assay with PI:3PS	10,000 assays	V1782	£4,448
ADP-Glo™ Kinase Assay with PIP2:3PS	1,000 assays	V1791	£539
ADP-Glo™ Kinase Assay with PIP2:3PS	10,000 assays	V1792	£4,448
PI3K (p110α/p85α), 20µg	200µl	V1721	£283
PI3K (p110α[E545K]/p85α), 20µg	200µl	V1731	£283
PI3K (p110α[H1047R]/p85α), 20µg	200µl	V1741	£283
PI3K (p110β/p85α), 20µg	200µl	V1751	£283
PI3K (p120γ), 20µg	200µl	V1761	£283
PI3K (p110δ/p85α), 20µg	200µl	V1771	£283
PIP2:3PS Lipid Kinase Substrate, 0.25mg	0.25ml	V1701	£204
PI:3PS Lipid Kinase Substrate, 0.5mg	0.5ml	V1711	£204

The non-mutant forms of PI3K enzymes can be purchased as part of the PI3K-Glo™ Class I Profiling Kit, which contains PI3Ks (5µg each), PIP2 substrate and the ADP-Glo™ Kinase Assay. The lipid substrate is supplied as frozen small unilamellar vesicles. A substrate composed of phosphatidylinositol (PI) and PS at a 1:3 ratio is also available, which is recognised by the majority of family members and provides a universal PI lipid kinase substrate.

GloMax® Multimode Plate Readers

Ready for your research

GloMax multimode plate readers are state-of-the-art instruments offering luminescence, fluorescence and absorbance detection capabilities, with preloaded protocols and touch screen functionality. Promega's GloMax Systems offer superior performance for a wide array of assays.



Easy to use

The intuitive touch-screen display, preloaded protocols and automatic instrument gain adjustments make it simple to produce data and analyse results.

Integrated with Promega assays

Optimised, preloaded Promega protocols for kinase assays and more are part of the GloMax® Systems Software; no need to optimise instrument settings.

Superior performance

Detect as little as 1 attomole of ATP or 1.5 zeptomoles of luciferase. Dynamic range of >9 logs extends the linear range of your assay and low cross-talk between wells gives better, more usable results.

World-class service and support

GloMax Systems come with a comprehensive one-year standard warranty, and our expert service team is available to answer your questions about the instrument or chemistries. In addition, we offer a full line of service products, including Installation and Operation Qualification (IQ/OQ), preventative maintenance and service agreements.

Ordering information

Product	Capability	Cat.No
GloMax® Discover System	Luminescence, Fluorescence Intensity, UV-Visible Absorbance, Filtered Luminescence, BRET and FRET	GM3000
GloMax® Explorer System, Fully Loaded	Luminescence, Fluorescence, Visible Absorbance	GM3500
GloMax® Explorer System	Luminescence, Fluorescence	GM3510
GloMax® Navigator System	Luminescence	GM2000

For more information or to request a demo, please visit:
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