PhosphoSens® Cell Lysate Activity Assay Format

JNK Assay Validation Using the AQT1196 Selective Sensor Peptide Substrate

HGNC Name: MAPK8 (JNK1), MAPK9 (JNK2), and MAPK10 (JNK3)
Long Names: c-**J**un **N**-terminal **K**inase, **M**itogen-**A**ctivated **P**rotein **K**inase (MAPK)

INTRODUCING A NEW INITIATIVE FROM OUR DIRECT-TO-PHASE II NIH/NCI SBIR AWARD ($2M):

PhosphoSens-Lysate

PhosphoSens-Lysate assays are here!

Experience the Benefits of Running Kinase Activity Assays in Complex Samples

Study Kinase Enzyme Activity Where Biology Happens

Simply Lyse Cells & Read
No Fractionation Needed

PhosphoSens-Lysate Assays transform kinase activity measurement by enabling continuous, real-time kinetic analysis directly in cell and tissue lysates. This innovative approach provides key insights into your target's native environment throughout the drug discovery pipeline.

NEW PRODUCTS **– Available Now for ERK1/2, AKT1/2/3, GSK3α/β, JNK1-3, and DNA-PK**

COMING SOON **– From our active R&D pipeline of selective sensor peptide substrates for:**
p38A/B/G/D, CDK1-3/5, CDK4/6, MEK1/2, SGK1/2/3, and PIM1/3.

PhosphoSens-Lysate Assays

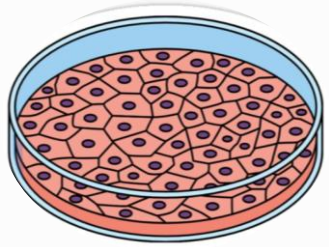
A Simple & Powerful Solution to a Complex Problem

AssayQuant® TECHNOLOGIES INC.

1. Grow Cells

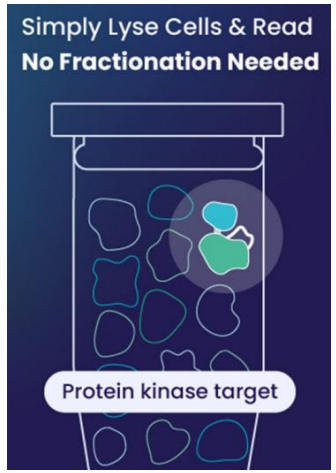
(+/- Pathway Activation)

or Access Tissues



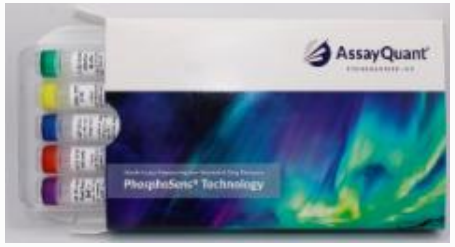
2. Harvest

ADD *PhosphoPreserve*
Lysis Buffer + Protease & Phosphatase Inhibitors



Study Kinase Enzyme Activity Where Biology Happens

We have combined innovative PhosphoSens detection technology, invented at MIT, with high-throughput peptide synthesis methods to design sensor peptide substrates that are highly selective for the target of interest. This development integrates the advantages of the PhosphoSens platform (activity-based, direct, homogeneous, continuous/kinetic, quantitative) with the enabling capability of measuring endogenous target kinase activity in unfractionated cell or tissue lysates.



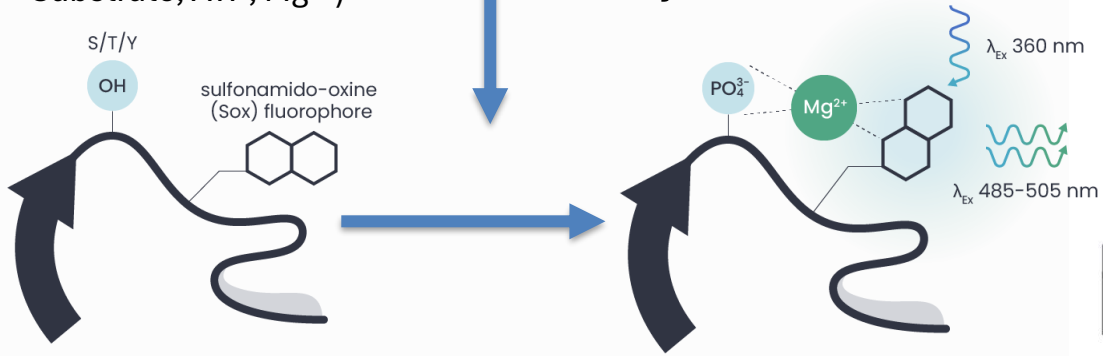
Easy-to-use Kits & Bulk Sensor Peptide

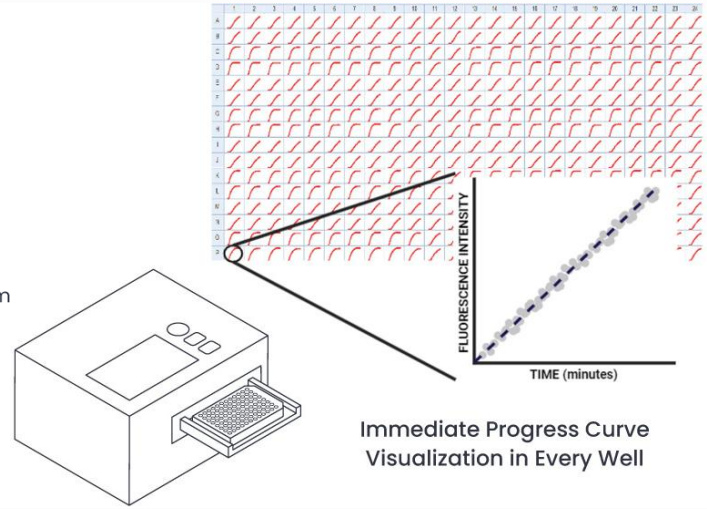
(Native substrates, stable, scalable, excellent lot consistency)

3. Prepare Reaction Mix

(PhosphoSens Sensor Peptide Substrate, ATP, Mg^{2+})

ADD Lysate & Read



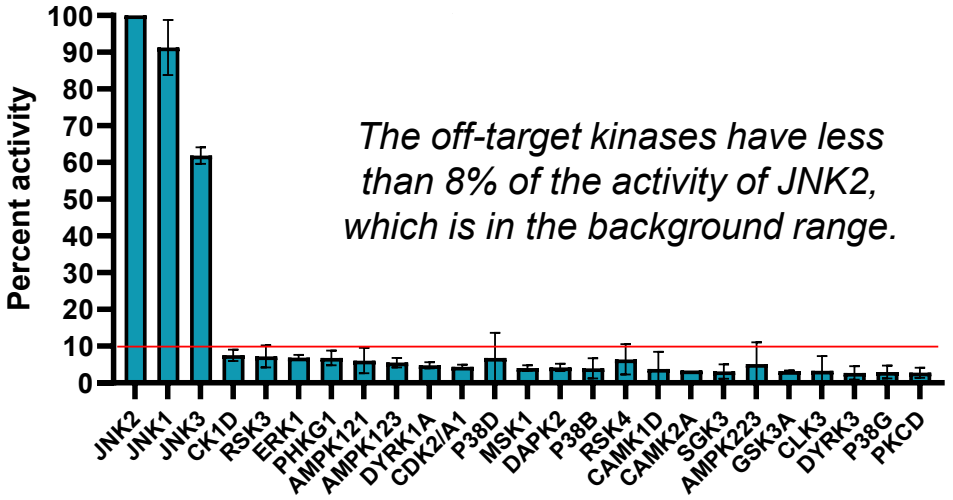


Immediate Progress Curve Visualization in Every Well

Selectivity of AQT1196 for JNK1/2/3 Determined with AQT's Kinome Profiling Service

AssayQuant TECHNOLOGIES INC.

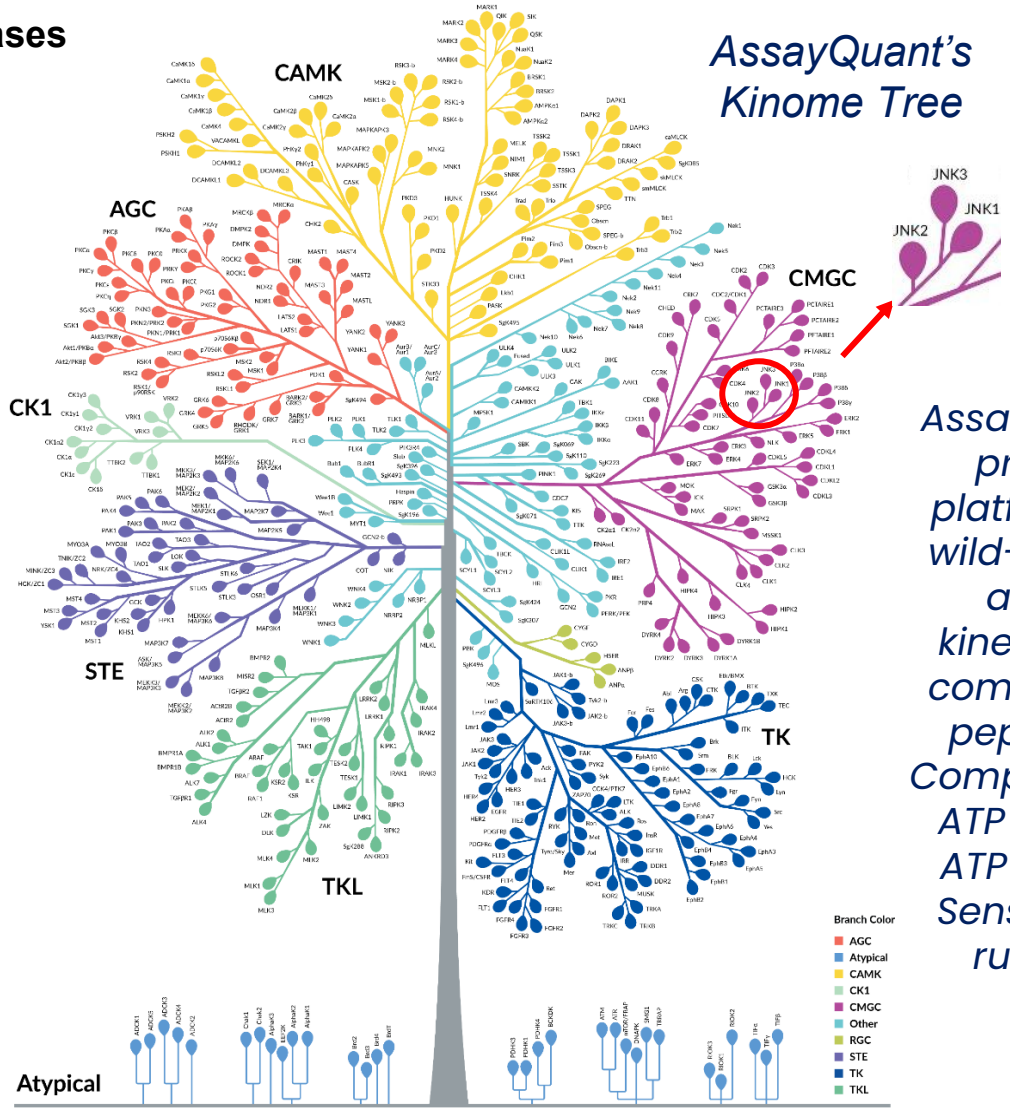
Top 25 Kinases from Kinome Profiling with 411 Wild-type Kinases



The off-target kinases have less than 8% of the activity of JNK2, which is in the background range.

#	Kinase target	Kinase Group	AQT1196, μM	Enzyme, nM	RFU/pmol/min	% Activity
1	JNK2	CMGC	10.0	4.0	**636.7**	100.0
2	JNK1	CMGC	10.0	4.0	**546.9**	85.9
3	JNK3	CMGC	10.0	5.0	**395.2**	62.1
4	CK1D	CK1	10.0	1.0	**48.5**	7.6
5	RSK3	AGC	10.0	1.0	**47.2**	7.4
6	ERK1	CAMK	10.0	0.4	**44.2**	6.9
7	PHKG1	CAMK	10.0	1.7	**42.4**	6.7
8	AMPK121	CAMK	10.0	1.0	**37.0**	5.8
9	AMPK123	CAMK	10.0	0.2	**34.4**	5.4
10	DYRK1A	CMGC	10.0	2.0	**30.9**	4.9
11	CDK2/A1	CMGC	10.0	0.3	**28.2**	4.4
12	P38D	CMGC	10.0	0.4	**26.5**	4.2
13	MSK1	AGC	10.0	1.0	**26.3**	4.1
14	DAPK2	CAMK	10.0	2.4	**26.2**	4.1
15	P38B	CMGC	10.0	0.8	**23.8**	3.7

AssayQuant's Kinome Tree



AssayQuant's Kinome profiling service platform features 411 wild-type kinases. All assays are run kinetically to assess compound or sensor peptide selectivity. Compounds are run at ATP K_m and/or 1 mM ATP (physiological). Sensor peptides are run at 1 mM ATP.

Outline for this Study

PhosphoSens-Lysate Assay Validation Using the AQT1196 Sensor Peptide Substrate

Lysate Source:

HEK293 cells (passage 17) +/- 3 µM Anisomycin (MedchemExpress, HY-18982; Resuspended in 100% DMSO)

Reference Compound Information:

JNK-IN-8 (JNK Inhibitor XVI - Cayman Chemical, Item No. 18096)

Experimental Validation at AssayQuant:

Lysate from HEK293 cells (+/- 3 µM Anisomycin for 40 minutes, as a stress stimulus, to activate JNKs)

Phosphopeptide Control (AQT1232) and comparison to phosphorylation of AQT1196 sensor peptide with recombinant JNK1 (Thermo Fisher, Part # PV3319)

AQT1196 sensor peptide substrate K_m determination

DMSO Tolerance Test

Reference Compound IC_{50} Determinations with JNK-IN-8.

Preparation of Cell Lysates from HEK293 Cells Treated +/- Anisomycin to Activate JNKs

1) HEK293 cells (passage 18) were plated in a 96-well tissue culture-treated plate and incubated for 48 hours at 37°C in EMEM medium supplemented with 10% FBS (ThermoFisher, A56708-01) and 1% Penicillin-Streptomycin (ThermoFisher, 15140122) in a 5% CO2 atmosphere.
2) Cells were washed with PBS, then incubated with culture medium containing 0.1% FBS (serum-starved to make the cells quiescent) and 1% Penicillin-Streptomycin for 24 hours. Subsequently, buffer (negative control) or Anisomycin (MeChemExpress, HY-18982; Resuspended in 100% DMSO) was added directly to the medium to a final concentration of 3 µM, and the cells were incubated for 40 minutes.
3) After the treatment, the culture medium was removed, the cells were washed with cold 1X PBS and then lysed with Cell Extraction Buffer (CEB) (see recipe below) for 15 minutes. The assay reaction mix (see next slide) and AQT1196 were added to lysates in each well to assess JNK activity.

Cell Extraction Buffer (CEB) with Protease and Phosphatase Inhibitors:

- 50 mM HEPES, pH 7.4
- 150 mM NaCl
- 2 mM EGTA
- 1 mM DTT
- 1% Triton X-100
- PhosphoPreserve Phosphatase Inhibitor Cocktail - 1, 100X
- PhosphoPreserve Phosphatase Inhibitor Cocktail - 3, 60X
- PhosphoPreserve Protease Inhibitor Cocktail, 60X

Titration of Cell Number and Lysate from HEK293 Cells +/-Anisomycin (3 µM) to Activate JNKs

Growth of HEK293 Cells, Treatment +/- Anisomycin, Cell Extraction, & Determination of JNK Activity with AQT1196 is Performed in the Same 96-well Plate

AssayQuant TECHNOLOGIES INC.

Reaction Conditions and Set Up

Reaction Conditions:

54 mM HEPES, pH 7.5
1 mM ATP
1.2 mM DTT
0.012% Brij-35
1% glycerol
0.2 mg/ml BSA
0.54 mM EGTA
10 mM $MgCl_2$
15 µM AQT1196 sensor peptide

JNK Enzyme:

- **Cell Lysate from titration of HEK293 cells:**
  - See below

Cell Plating and Protein Determination:

HEK293 Cells were seeded in a 96-well plate (6 point, 2-fold serial dilution) at 2.5k, 5.0k, 10k, 20k, 40k, and 80k cells/well

Total protein was determined by the Bradford method: 0.1, 0.3, 2.3, 6.1, 16, and 26 µg of protein/well

Reaction Set Up:

- To **20 µL of Cell Lysate** in the 96-well plate
- Add **78 µL Reaction Mix with AQT1196, ATP & DTT**
- Add **2 µL of 50X tool compound** in DMSO or DMSO control
- To obtain **100 µL** Final reaction volume

Notes:

Reaction was run at 30 ˚C for 240 minutes in Falcon® 96-well White Flat Bottom TC-treated Microplate (Corning Cat. #353296) at 100 µL final well volume after sealing using optically-clear adhesive film (TopSealA-Plus plate seal, PerkinElmer [Cat. #6050185]) in a Biotek Synergy Neo 2 microplate reader with excitation (360 nm) and emission (485 nm) wavelengths.

1X Enzyme Dilution Buffer (EDB) is used to dilute enzyme and for the blank. Composition is 20 mM HEPES, pH 7.5, 0.01% Brij-35, 5% Glycerol, 0.5 mM EGTA, 1 mM DTT, 1 mg/ml Bovine Serum Albumin.

Recombinant JNK1 Activity with AQT1196 and Comparison to AQT1232 Phospho-Control

Reaction Conditions and Set Up

Reaction Conditions:

54 mM HEPES, pH 7.5

1 mM ATP

1.2 mM DTT

0.012% Brij-35

1% glycerol

0.2 mg/ml BSA

0.54 mM EGTA

10 mM $MgCl_2$

15 µM **Sensor peptides:**

- AQT1196 sensor peptide substrate
- AQT1232 phosphopeptide control

5 nM JNK1, full-length recombinant (Thermo Fisher, Part # PV3319)

Reaction Set Up:

20 µL Reaction Mix with AQT1196 or AQT1232, ATP, DTT
15 minutes incubation at 30°C (in the reader)
5 µL of 5x enzyme or EDB with lysate buffer
25 µL Final reaction volume

Reaction was run at 30 ˚C for 240 minutes in either Corning, low volume 384-well, white flat-bottom polystyrene NBS microplates (Cat. #3824) at 20 or 25 µL final well volume or in in PerkinElmer, ProxiPlate-384 Plus, white shallow well microplates (Cat. #6008280) at 20 µL final well volume after sealing using optically- clear adhesive film (TopSealA-Plus plate seal, PerkinElmer [Cat. #6050185]) in a Biotek Synergy Neo 2 microplate reader with excitation (360 nm) and emission (485 nm) wavelengths.

Notes:

Enzyme Dilution Buffer (EDB): 20 mM HEPES, pH 7.5, 0.01% Brij-35, 5% Glycerol, 0.5 mM EGTA, 1 mM DTT, 1 mg/ml Bovine Serum Albumin.

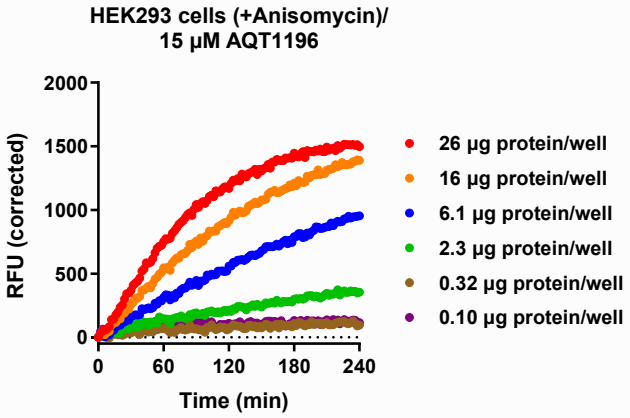
Titration of Cell Number and Lysate from HEK293 Cells +Anisomycin (3 µM) to Activate JNKs

Progress Curves, Reaction Rates & Linearity for JNK Activity with AQT1196

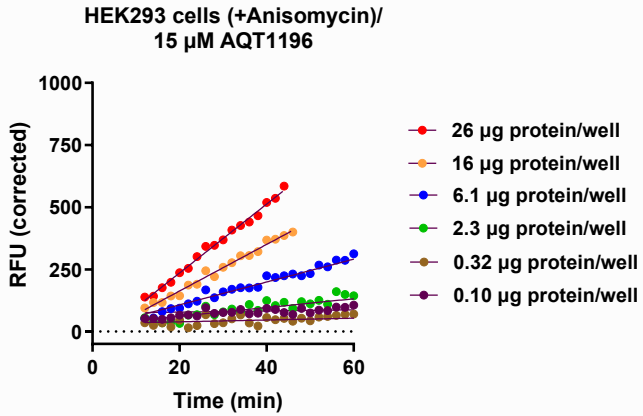
AssayQuant TECHNOLOGIES INC.

Progress Curves

Complete Time Course

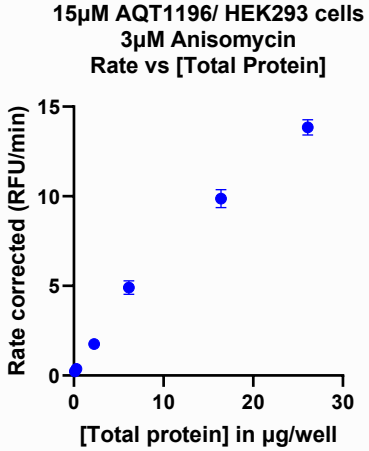


Linear Region

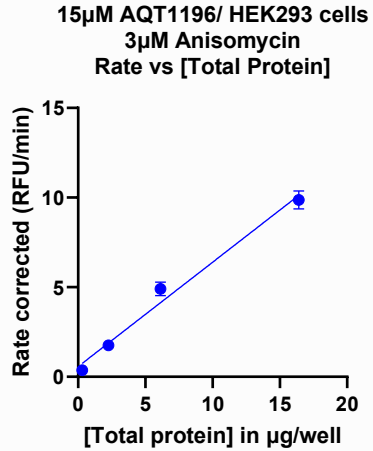


Assessment of Linearity

All Data



Linear Reaction



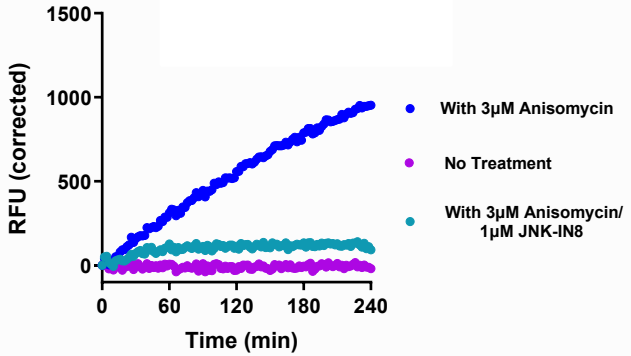
The JNK lysate activity assay is linear from 0.3 – 16 µg/well of crude protein (53-fold range), corresponding to 5k – 16k HEK293 cells seeded/well.

JNK Lysate Activity Assay with AQT1196

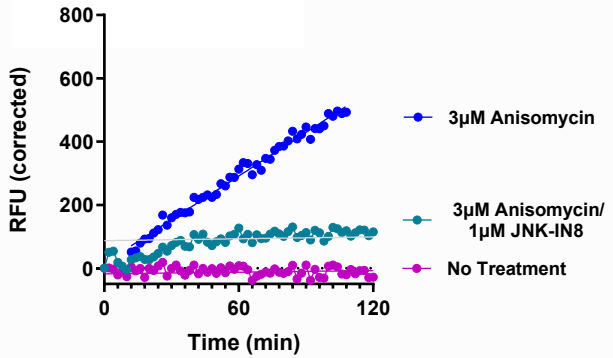
Activation of JNK in Lysates from HEK293 Cells ± Anisomycin

A. Crude Lysate Samples: 20k HEK293 cells (or 6.1 µg protein)/well with 15 µM AQT1196

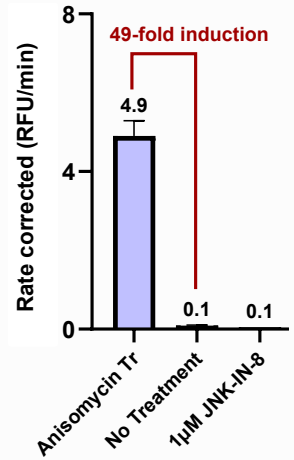
1) Full Time Course (0-240 min.)



2) Linear Range (4-120 min.)

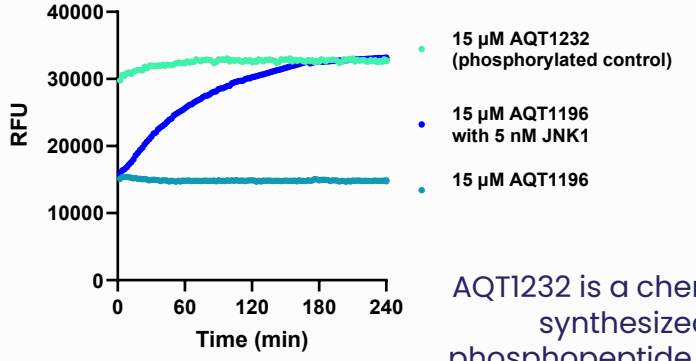


3) Histogram



B. Purified JNK1 & AQT 1232 Control

1) Full Time Course (0-240 min.)



AQT1232 is a chemically synthesized phosphopeptide control

A. Crude lysate samples: The AQT1196 sensor peptide was used to generate RFU Corrected values (Total – Background) for **1)** Full progress curve time course (0-240 min.), and **2)** Linear range (4-120 min.), and to determine the slope for each condition, which is the Reaction rate (RFU Corrected/min. +/- standard deviations) shown as a histogram in **3)**, highlighting a 49-fold activation of JNK kinase activity in HEK293 cell lysates treated with 3µM Anisomycin for 40 minutes. The signal was eliminated by adding the selective JNK1/2/3 inhibitor JNK-IN-8 (1 µM). The amount of activation depends on several factors, including cell type, serum concentration, and duration of the pre-incubation to make cells quiescent, and the activating stimulus's nature, concentration, and duration. These conditions can be varied to optimize JNK activity. The total amount of JNK1/2/3 protein can be determined by Western Blotting or an ELISA; however, with the short stimulation times typically used, these levels are not expected to change.

B.1. Purified recombinant JNK1 enzyme & AQT1107 Control: JNK1 enzyme (5 nM) fully phosphorylated the AQT1196 sensor peptide substrate by 240 min., as shown by convergence with the signal obtained with the AQT1232 phosphopeptide positive control (a flat horizontal line defining the maximum RFU with this sensor peptide). The signal with JNK1 enzyme was eliminated by adding the JNK-IN-8 inhibitor (1 µM). The signal with AQT1232 is used to convert RFU (Corrected) values to nmoles of product.

Sensor Peptide K_m Determination for AQT1196 Using Lysate from HEK293 Cells (20k/well) + Anisomycin

Reaction Conditions and Set Up

Reaction Conditions:

54 mM HEPES, pH 7.5

1 mM ATP

1.2 mM DTT

0.012% Brij-35

1% glycerol

0.2 mg/ml BSA

0.54 mM EGTA

10 mM $MgCl_2$

AQT1196 sensor peptide titration - 0, 0.20, 0.39, 0.78, 1.6, 3.1, 6.3, 13, 25, 50, and 100 µM.

Cell Plating and Protein Determination:

HEK293 Cells were seeded at 20k cells/well in 24 wells (12 wells in duplicate) of the 96-well plate

Total protein was determined by the Bradford method: 6.1 µg of protein/well

Reaction Set Up:

- To **20 µL Lysate** in the 96-well plate,
- Add **70 µL Reaction Mix with ATP, &DTT**
- Add **10 µL of 10X AQT1196**
- to obtain **100 µL** Final reaction volume

Notes:

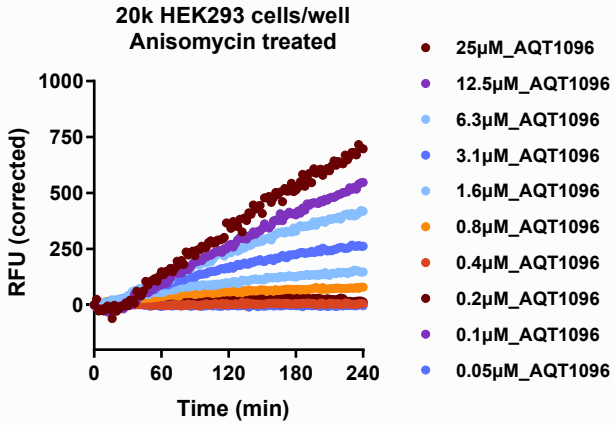
Reaction was run at 30 ˚C for 240 minutes in Falcon® 96-well White Flat Bottom TC-treated Microplate (Corning Cat. #353296) at 100 µL final well volume after sealing using optically-clear adhesive film (TopSealA-Plus plate seal, PerkinElmer [Cat. #6050185]) in a Biotek Synergy Neo 2 microplate reader with excitation (360 nm) and emission (485 nm) wavelengths.

1X Enzyme Dilution Buffer (EDB) is used to dilute enzyme and for the blank. Composition is 20 mM HEPES, pH 7.5, 0.01% Brij-35, 5% Glycerol, 0.5 mM EGTA, 1 mM DTT, 1 mg/ml Bovine Serum Albumin.

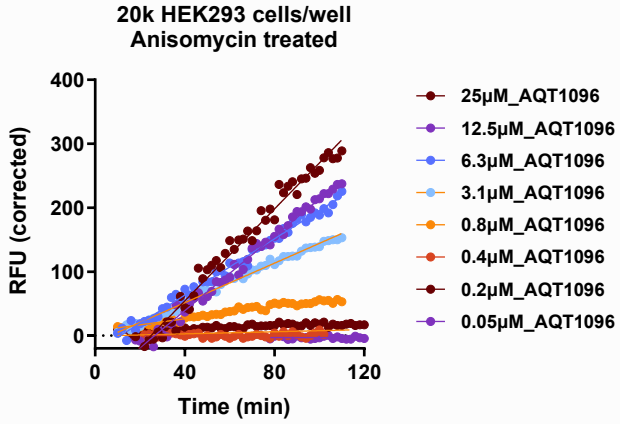
Sensor Peptide K_m Determination with AQT1196 Using Lysate from HEK293 Cells (20k/well) + Anisomycin

Titration Curves and K_m Plot and Table

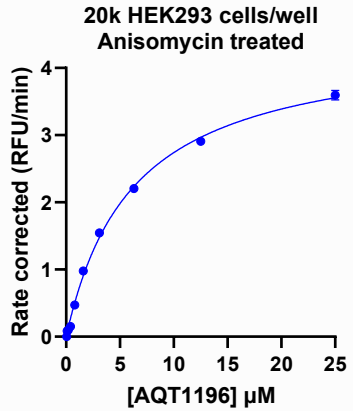
Sensor Peptide progress Curves



Sensor Peptide Linear range



Sensor Peptide K_m Plot



Michaelis-Menten	
Best-fit values	
Vmax	4.439
Km	6.206
Std. Error	
Vmax	0.1175
Km	0.4234
95% CI (asymptotic)	
Vmax	4.168 to 4.710
Km	5.229 to 7.182

The K_m value for AQT1196 is 6.2 μM.

DMSO Tolerance Test Using Lysate from HEK293 Cells (20k/well) + Anisomycin and AQT1196

AssayQuant TECHNOLOGIES INC.

Reaction Conditions:

54 mM HEPES, pH 7.5

1 mM ATP

1.2 mM DTT

0.012% Brij-35

1% glycerol

0.2 mg/ml BSA

0.54 mM EGTA

10 mM $MgCl_2$

15 µM AQT1196 sensor peptide

Cell Plating and Protein Determination:

HEK293 Cells were seeded at 20k cells/well in 24 wells (12 wells in duplicate) of the 96-well plate

Total protein was determined by the Bradford method: 6.1 µg of protein/well

Reaction Set Up:

- To **20 µL Lysate** in the 96-well plate,
- Add **70 µL Reaction Mix with AQT1196, ATP, &DTT**
- Add **10 µL of 10X DMSO**
- to obtain **100 µL** Final reaction volume

Notes:

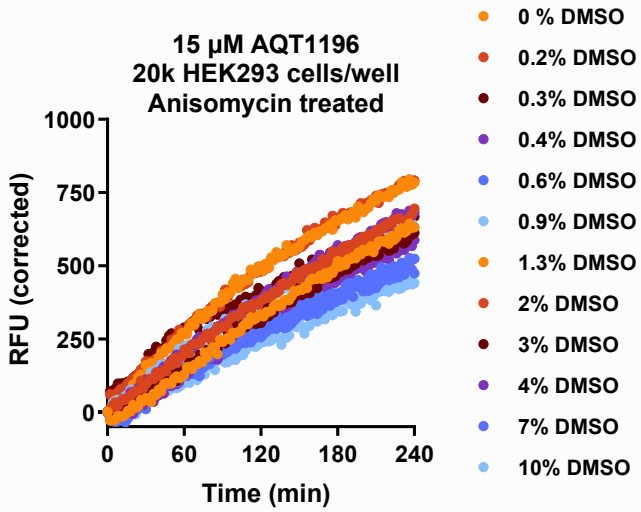
Reaction was run at 30 ˚C for 240 minutes in Falcon® 96-well White Flat Bottom TC-treated Microplate (Corning Cat. #353296) at 100 µL final well volume after sealing using optically-clear adhesive film (TopSealA-Plus plate seal, PerkinElmer [Cat. #6050185]) in a Biotek Synergy Neo 2 microplate reader with excitation (360 nm) and emission (485 nm) wavelengths.

1X Enzyme Dilution Buffer (EDB) is used to dilute enzyme and for the blank. Composition is 20 mM HEPES, pH 7.5, 0.01% Brij-35, 5% Glycerol, 0.5 mM EGTA, 1 mM DTT, 1 mg/ml Bovine Serum Albumin.

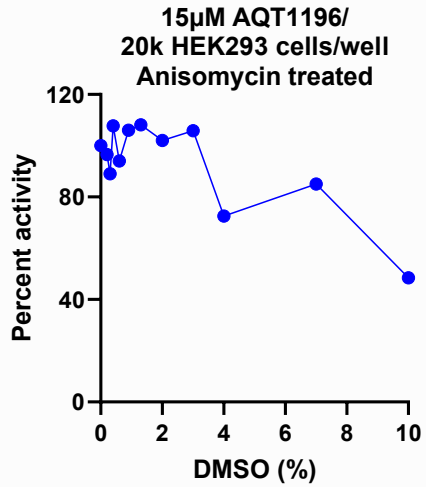
DMSO Tolerance Test Using Lysate from HEK293 Cells (20k/well) + Anisomycin and AQT1196

Titration Curves and Inhibition Plot

Complete Progress Curves



Reaction Rate vs [DMSO] Plot



No significant loss in JNK activity was observed up to 3% DMSO.
A final concentration of 2% DMSO will be used to assess inhibitor potency.

IC_{50} Determination using AQT1196 in 96-well plates with Lysates from HEK293 Cells (20k/well)

Reaction Conditions and Set Up

Reaction Conditions:

54 mM HEPES, pH 7.5

1 mM ATP

1.2 mM DTT

0.012% Brij-35

1% glycerol

0.2 mg/ml BSA

0.54 mM EGTA

10 mM $MgCl_2$

15 µM AQT1196 sensor peptide

Tool Compound - JNK-IN-8 was titrated with 3-fold dilutions in 100% DMSO at 50X the final concentrations and then diluted 50-fold into the assay for final concentrations from 0-10 µM in 2% DMSO.

Reaction Set Up:

- To **20 µL Lysate** in the 96-well plate,
- Add **78 µL Reaction Mix with AQT1196, ATP, &DTT**
- Add **2 µL of 50X serially diluted JNKJ-IN-8 in 100% DMSO**
- to obtain **100 µL** Final reaction volume

Notes:

Reaction was run at 30 ˚C for 240 minutes in Falcon® 96-well White Flat Bottom TC-treated Microplate (Corning Cat. #353296) at 100 µL final well volume after sealing using optically-clear adhesive film (TopSealA-Plus plate seal, PerkinElmer [Cat. #6050185]) in a Biotek Synergy Neo 2 microplate reader with excitation (360 nm) and emission (485 nm) wavelengths.

1X Enzyme Dilution Buffer (EDB) is used to dilute enzyme and for the blank. Composition is 20 mM HEPES, pH 7.5, 0.01% Brij-35, 5% Glycerol, 0.5 mM EGTA, 1 mM DTT, 1 mg/ml Bovine Serum Albumin.

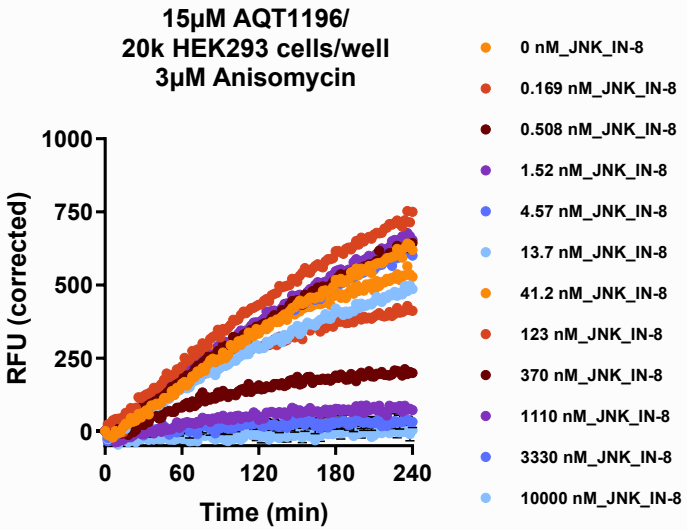
Cell Plating and Protein Determination:

HEK293 Cells were seeded at 20k cells/well in 24 wells (12 wells in duplicate) of the 96-well plate

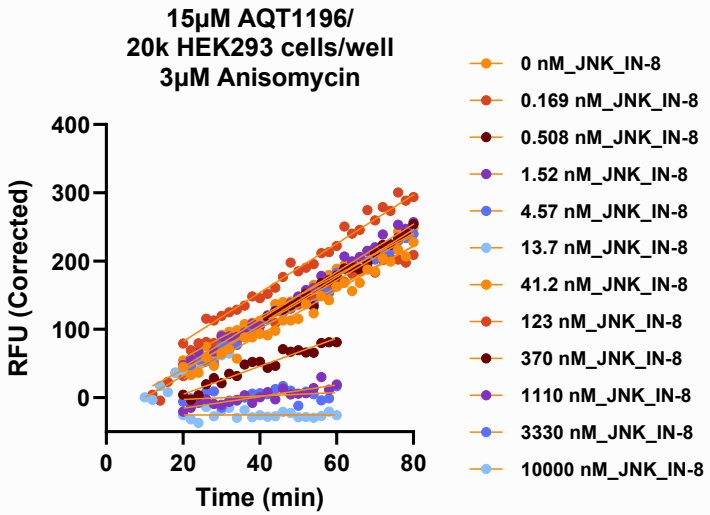
Total protein was determined by the Bradford method: 6.1 µg of protein/well

IC_{50} Determination using AQT1196 in 96-well plates with Lysates from HEK293 Cells (20k/well)

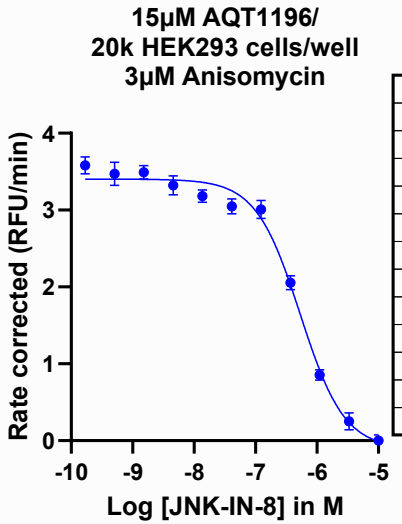
Full Progress Curves



Progress Curves (Linear Region)



IC_{50} Curve

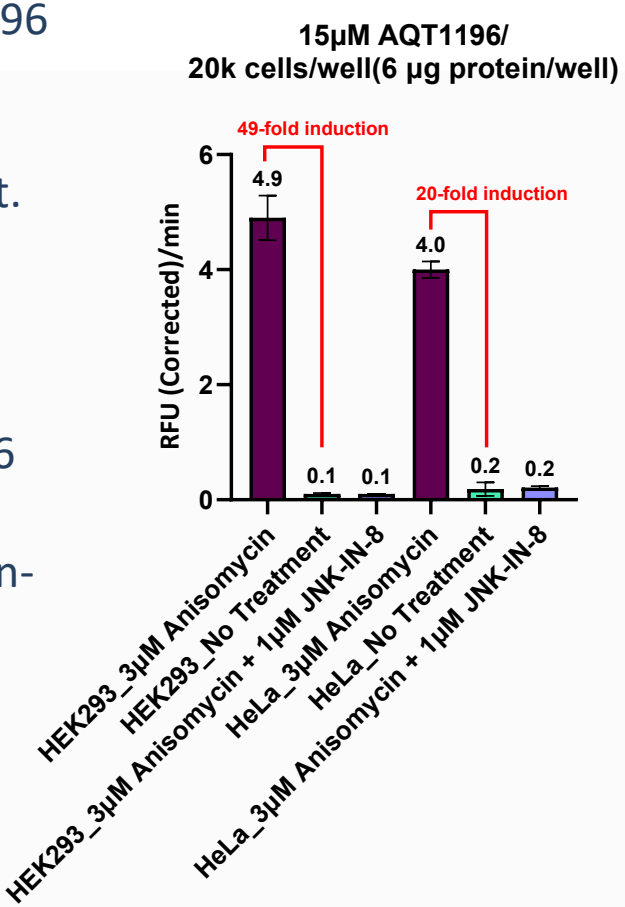


Best-fit values	
Bottom	-0.1095
Top	3.403
LogIC50	-6.273
HillSlope	-1.207
IC50	5.332e-007
Span	3.512
Std. Error	
Bottom	0.1721
Top	0.06462
LogIC50	0.06570
HillSlope	0.1852
Span	0.1961

The IC_{50} value for JNK-IN-8 in lysate from anisomycin-treated HEK293 cells is 533 nM, which is close to the value of 290 nM reported by Peterson et al. (Biochemistry 2014, 53, 5771-5778) with HeLa cell lysates

Summary

- The PhosphoSens-Lysate Activity Assay for JNK1-3 using the selective sensor peptide AQT1196 demonstrates a robust and physiologically relevant assay that provides a functional assessment of endogenous JNK activity with all the cellular components and signaling complexes. This JNK activity assay is direct, highly quantitative, and in an easy-to-use format.
- Results include:
  - Anisomycin treatment resulted in vigorous activation of JNK activity in HEK293 (49-fold) and HeLa (20-fold) cells.
  - JNK activity with lysates from Anisomycin-treated HEK293 cells was linear from 0.3 to 16 µg protein/well, a 53-fold linear range.
  - The Sensor peptide substrate AQT1196 has a K_m of 6.5 µM when tested with Anisomycin-treated HEK293 cell lysates.
  - 97% of AQT1196 phosphorylation in lysates from Anisomycin-treated HEK293 cells is inhibited by the reference compound for JNK (JNK-IN-8, 1 µM).
  - The IC_{50} value for JNK-IN-8 in lysates from anisomycin-treated HEK293 cells with AQT1196 was 533 nM.



AQT1196 enables selective and precise quantitation of JNK1/2/3 activity with different cell types (demonstrated for HEK293 and HeLa cells), providing a powerful tool for evaluating pathway activation and inhibition in complex samples from normal and disease states

Company & Technology Supporting Slides

AQT Senior Scientific Team

Experienced & Diverse Team from Leading Institutions

AssayQuant TECHNOLOGIES INC.

Dr. Bill Radany
CEO & Head of Business Development

Dr. Erik Schaefer
Co-Founder, CSO

Dr. Barbara Imperiali
Co-Founder & CTO, MIT Faculty

Dr. Earl May
Senior Director, Discovery Technologies (DT)

Dr. Eric Berg
Director of Peptide Operations

Dr. Daniel Urul
Senior Scientist, DT

Dr. Venky Nemmara
Senior Scientist, DT

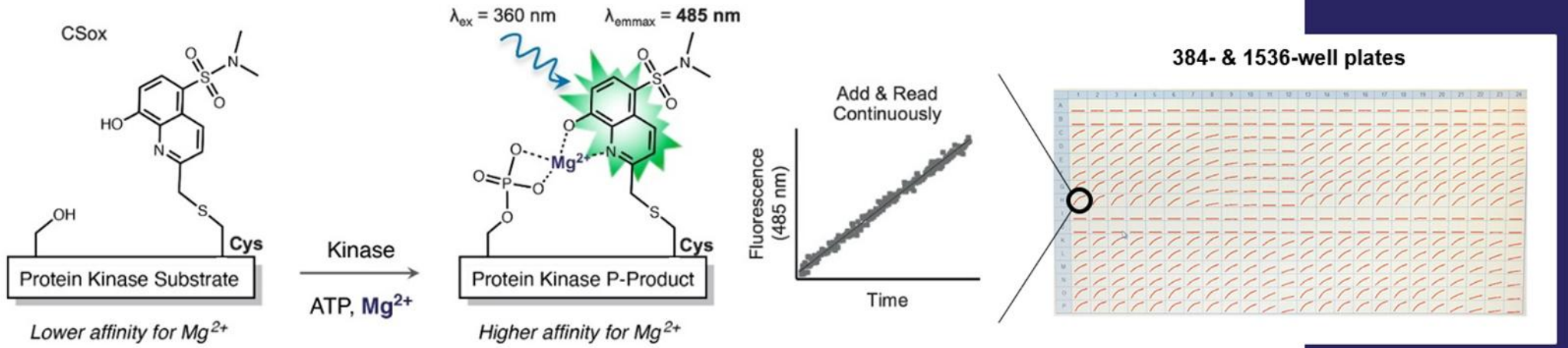
- We apply our >200 years of collective experience in kinase biology, drug or assay development, chemical-biology and automation to solve critical problems in target biology and drug development.
- AQT services allow us to evaluate drugs in diverse ways, delivering high-quality data combined with strong communication to enable discovery

Susan Cornell-Kennon
Senior Scientist, DT

Dr. Satish Pimrale
Business Development Leader

gsk | Pfizer | astellas | Bristol Myers Squibb | BIACORE | danaher | Caliper LifeSciences | PerkinElmer | ThermoFisher SCIENTIFIC | Merck KGaA Darmstadt, Germany | Promega

Sensor Peptide Substrates for Continuous (Kinetic) Monitoring of Protein Kinase Activity



- Uses chelation-enhanced fluorescence via sulfonamido-oxine (Sox) chromophore, invented by Barbara Imperiali (MIT).
- 10 patents with 1 pending, exclusively licensed from MIT. Rich trade secret portfolio
- Sox is small, minimally hydrophobic, and neutral
- Assay is direct, homogeneous, and kinetic (continuous)
- Can use a wide range of ATP concentrations (Low µM, ATP K_m or mM [physiological])
- Runs on commonly available readers with any plate type (96-, 384- or 1536-well)

Simple, Powerful & Flexible!

AssayQuant® TECHNOLOGIES INC.

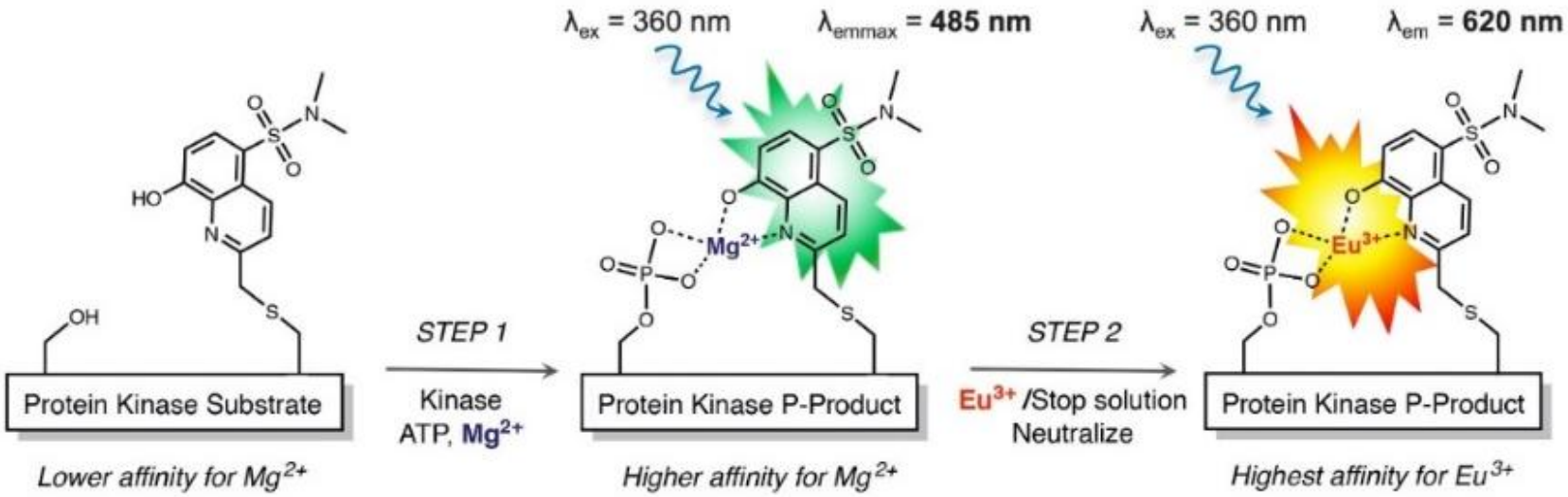
a full progress curve in every well

Ideal for quantitative assessments including Profiling, Potency & MOA

PhosphoSens®

Replacing Mg^{2+} with Eu^{3+} Create

PhosphoSens®- Red



- Sox sensitizes Europium (Eu^{3+}) luminescence for time-resolved fluorescence (TRF) with 620 nm emission and data acquisition delay, which eliminates compound interference
- Run as an Endpoint format; ideal for high-numbers of tests for HTS and SAR
- Patent approved in 2020

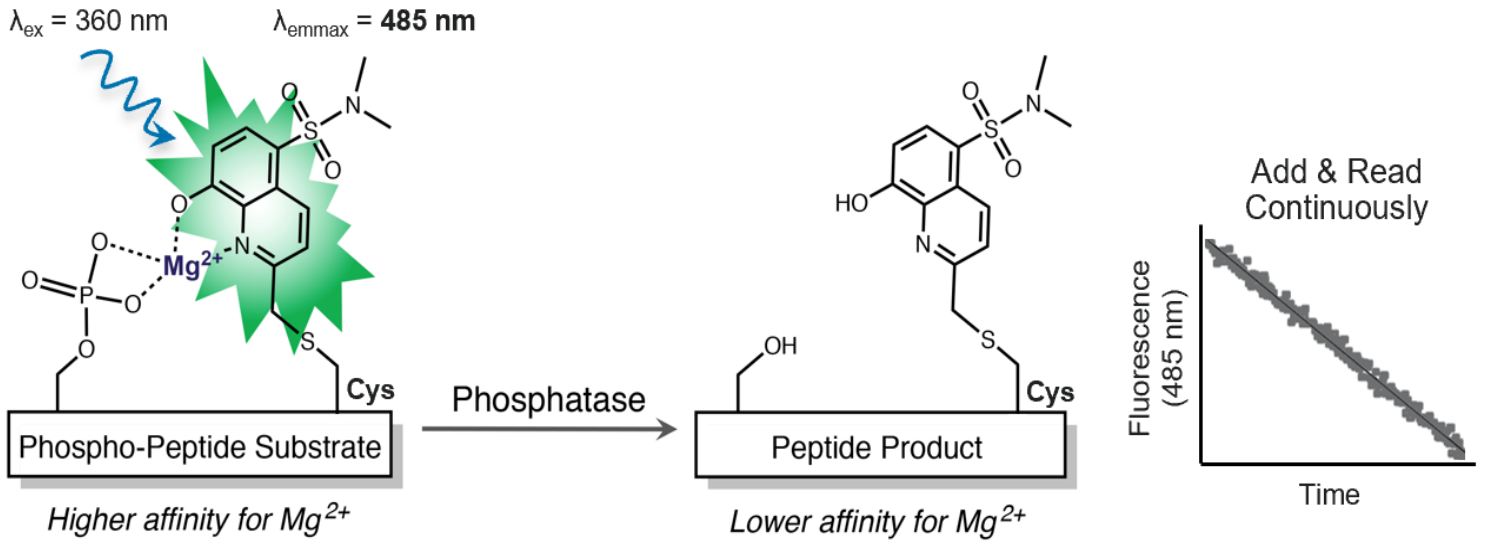
Eliminates autofluorescence while using the same small fluorophore

AssayQuant® TECHNOLOGIES INC.

Ideal for:

- HTS
- SAR

Same Sox-based Detection Platform Enables Continuous Monitoring of **Protein Pho**



- Same mechanism - ChEF (Mg^{2+}) for kinetic assays and Eu^{3+}/TRF for Endpoint (Red), as for protein kinases
- Uses **CSox-based phosphopeptide substrates** derived from physiological targets to monitor loss of signal
- Covered by 10 patents (with 1 pending) and trade secret portfolio

Increasing requests for tyrosine & serine/threonine phosphatases

AssayQuant TECHNOLOGIES INC.

Kinetic and Endpoint

One Technology, Two Fit-for-Purpose Formats

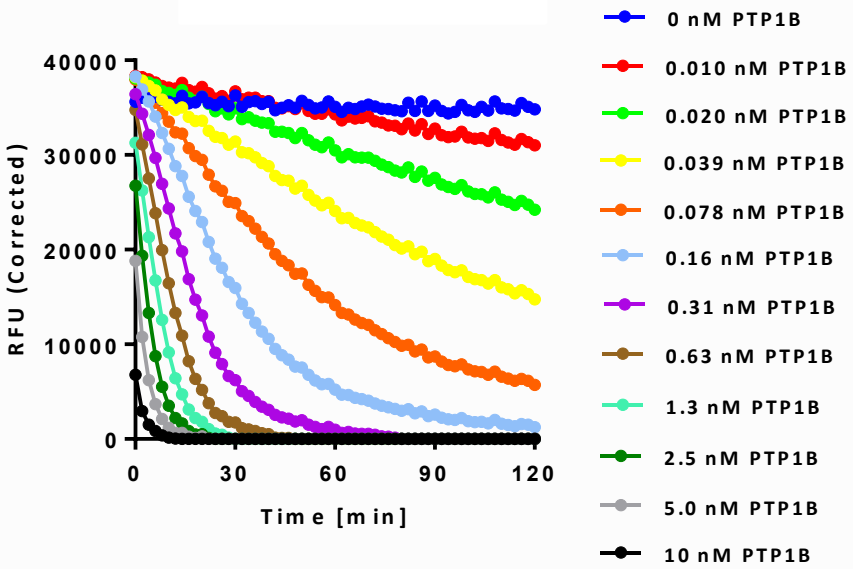
Expanding menu (currently 34 of the 190 Protein Phosphatases)

Analysis of **PTP1B** & **PTPN2** Tyrosine Phosphatases

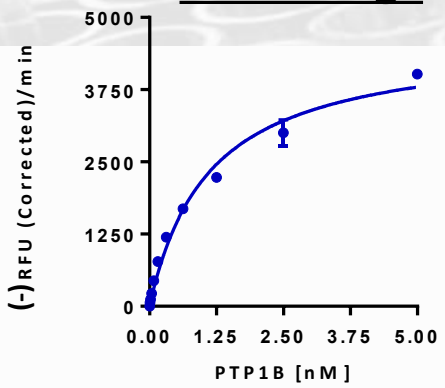
Optimized *PhosphoSens* Phosphopeptide Substrates

AssayQuant TECHNOLOGIES INC.

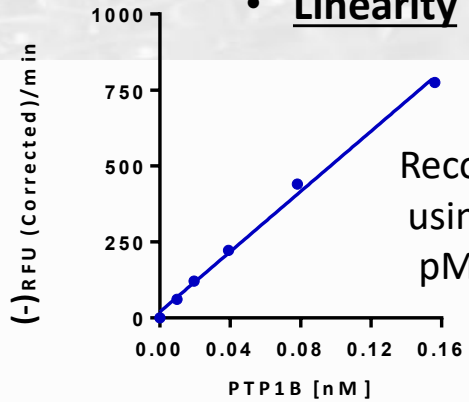
Progress Curves & Linearity for PTP1B with AQT0266 Sensor Peptide, Net Signal



Total Range

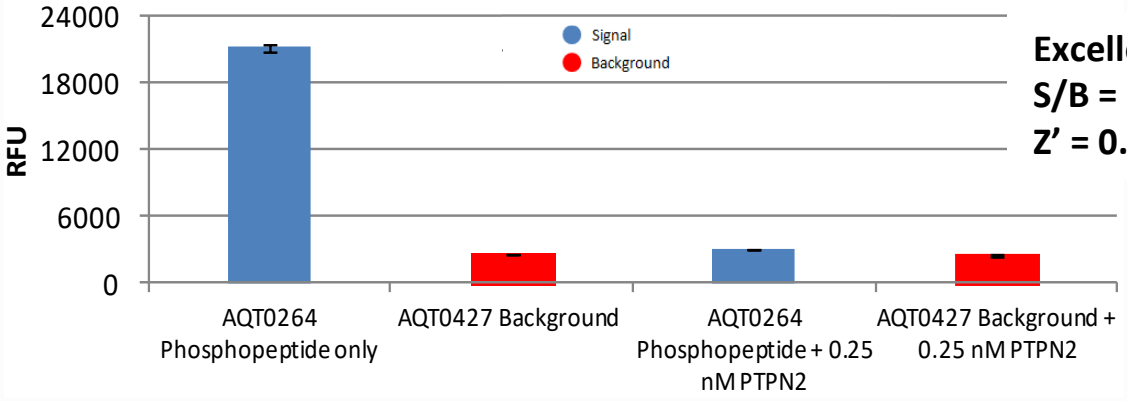


- **Linearity**



Recommend using 20-75 pM PTP1B

Total & Background for PTPN2 with AQT0264 in PhosphoSens®-Red



Excellent Assay
S/B = -7.1
Z' = 0.94 (n=3)

High-sensitivity & physiologically-relevant. Analysis of allosteric or substrate-competitive inhibitors

PhosphoSens® Platform

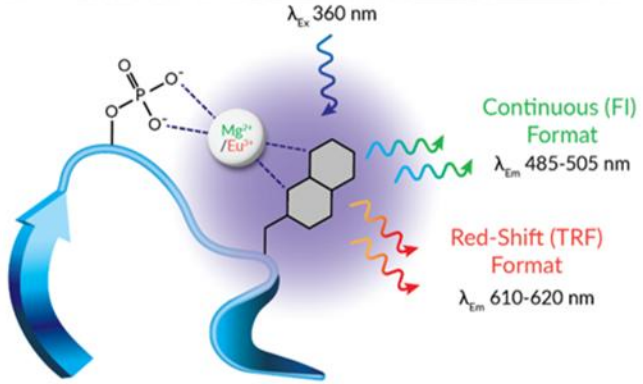
Enabling Analysis Across the Drug Development Workflow

AssayQuant TECHNOLOGIES INC.

96-, 384-, or 1536-well plates

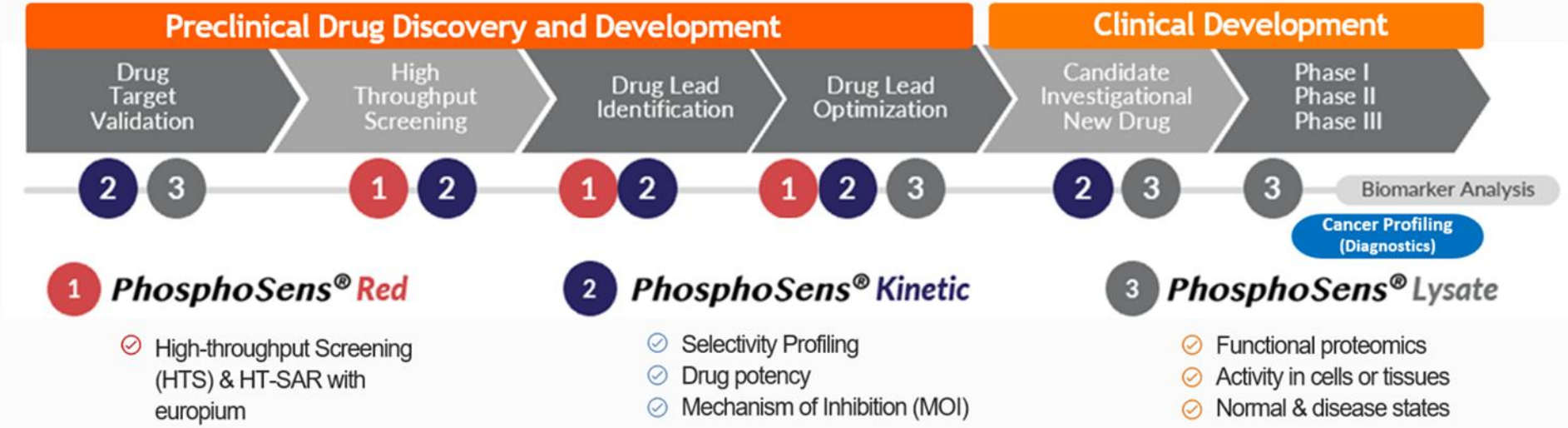
Any fluorescence microplate reader (kinetic mode)

Mix and read!



One Technology, Two Fit-for-Purpose Formats

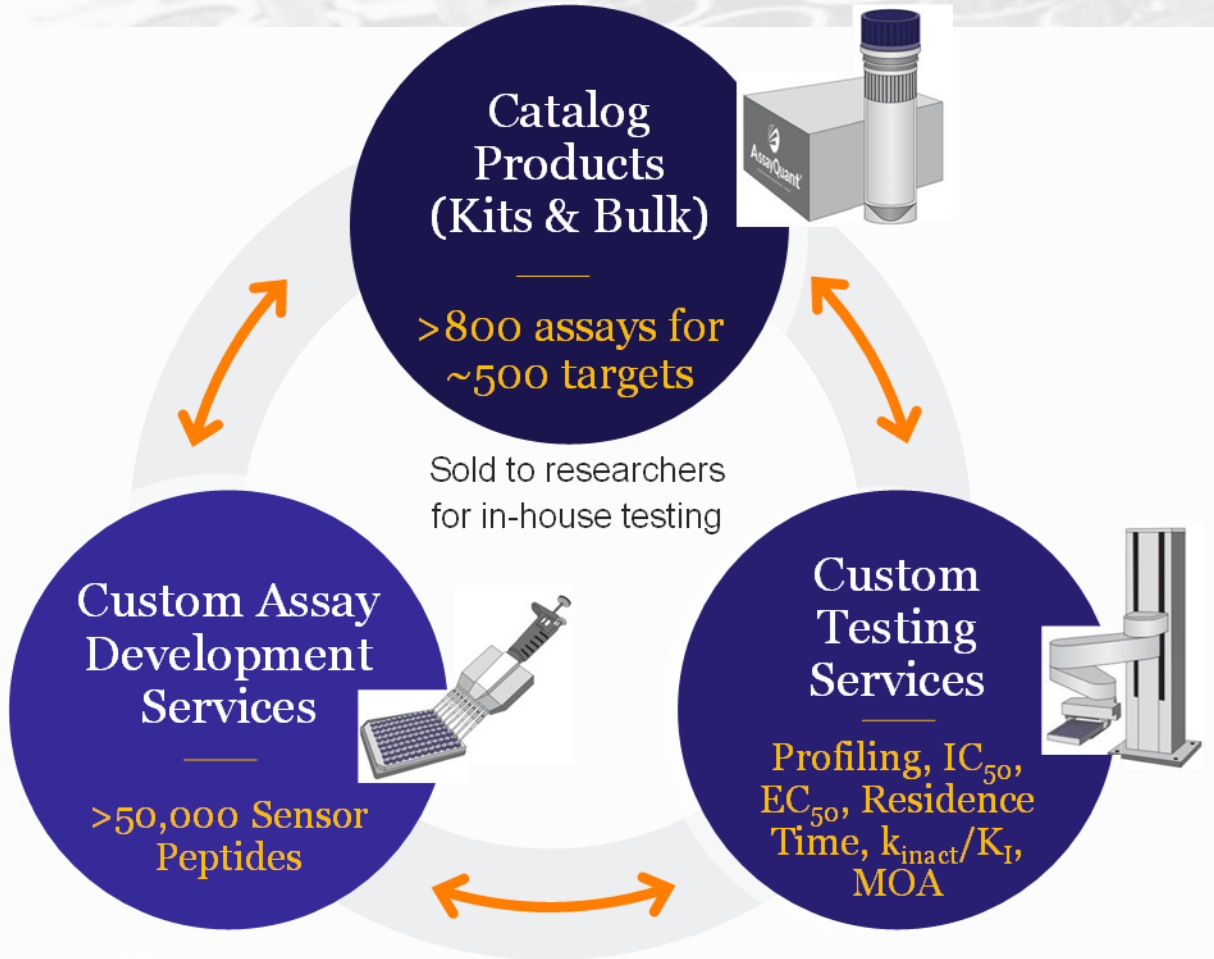
Kinetic and Endpoint



Accelerates progress and improves outcomes

Integration of Catalog Products & Services

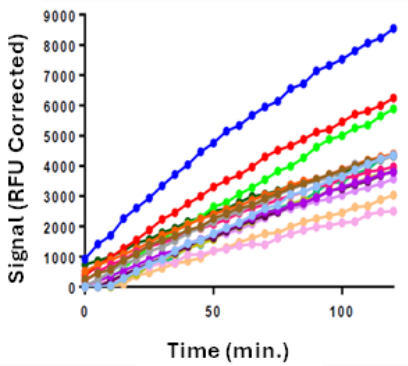
Multiple entry & transition points to address your needs



PhosphoSens® Platform Continuous Workflow Applications

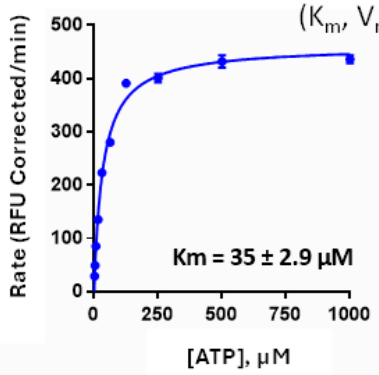
AssayQuant TECHNOLOGIES INC.

Assay Development



Enzyme Kinetics with Recombinant Proteins

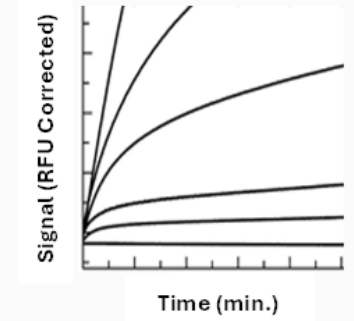
(K_m, V_{max}, k_{cat}, k_{cat}/K_m)



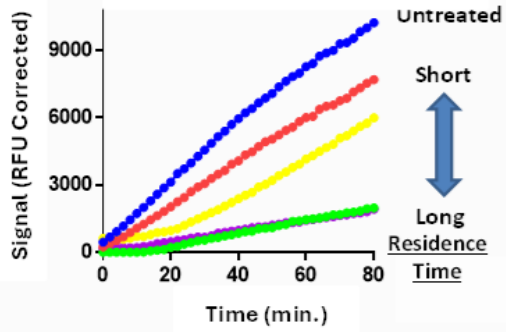
Fluorescence increase	[Km (µM)]	V_{max} (µmol.mg^{-1}.min^{-1})
3.5 - fold	0.01	1.8
3.9 - fold	0.69	2.5
4.4 - fold	1.2	1.3

Drug Potency (IC_{50}, K_i, Residence Time, k_{inact}/K_I, MOA, EC_{50})

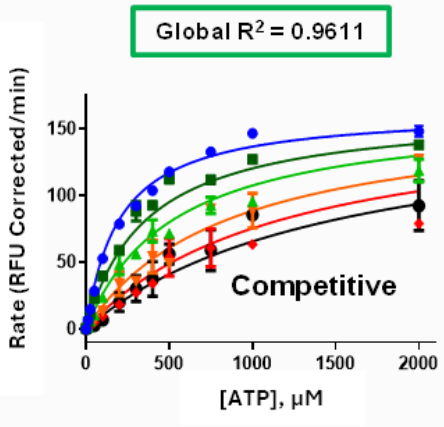
Time-Dependent Inhibition (TDI)



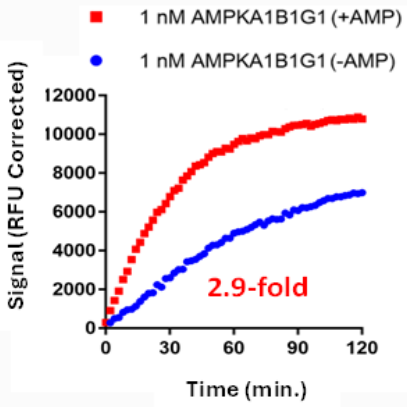
EGFR (T790M/C797S/L858R)



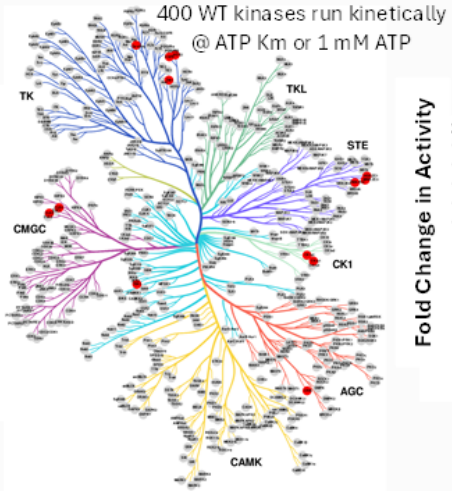
Mechanism of Inhibition (MOI)



Target Activation

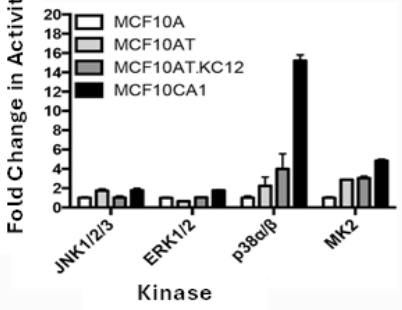


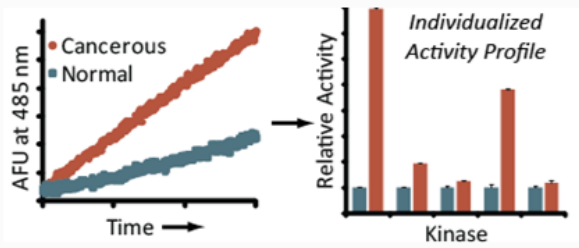
Kinome Profiling



Quantitative Enzyme Activity Measurements in Unfractionated Cell or Tissue Lysates

Direct-to-Phase II SBIR from NIH/NCI funded in August 2024





Crude lysate figures from Imperiali lab (MIT): Peterson *et al.* Biochemistry 2014, 53: 5771-5778; Stains *et al.* Chem Biol. 2012, 19(2): 210–217.

PhosphoSens® Platform Advantages:

- **Sensitive** and **continuous** (**kinetic**) format based on Chelation-enhanced fluorescence.
- **Homogeneous (add & read) and direct** measure of enzyme activity resulting in a complete **progress curve in every well** for each condition.
- Sensor peptides or lipids based on **physiologically-relevant** substrates.
- Run under **optimal enzyme conditions**, with physiological **Mg^{2+}, Mn^{2+} & Ca^{2+}** ions and **any ATP** concentration (1-2 mM = physiological; or at ATP K_m) or sample type (lysates via selective sensor peptides or IP-kinase assays).
- Determination of **initial reaction rate** from **linear** portion of curve provides **high accuracy & precision** (Z'>0.7) and enables assessment of time-dependent inhibitor or activator potency (IC_{50}, EC_{50}, k_{inact}/K_I, residence time).
- **Corrects for any compound autofluorescence** – **Kinetic:** Background signal doesn't change over time; **Red:** Uses Europium (Eu^{3+}) for time-resolved fluorescence (TRF) with a 100 µsec delay & 620 nm emission.
- **Rapid & predictable development** of new assays is achieved even with difficult targets using >30,000 sensor peptide panel for protein kinases or phosphatases and any DAG species for DGK assays.
- **Rigorous manufacturing** process for PhosphoSens® sensors provides excellent lot to lot consistency.
- **Works across entire drug development workflow** – Improving efficiency and performance

We apply our >200 years of collective experience in kinase biology, drug or assay development, and automation to address complex biology and evaluate drugs in diverse ways, delivering high-quality data to accelerate your programs.