# *PhosphoSens*® Cell Lysate Activity Assay Format

ERK1/2 (MAPK3/1) Assay Validation Using the AQT1076 Sensor Peptide

HGNC Name: MAPK1 (ERK2) and MAPK3 (ERK1)
Long Names: **E**xtracellular Signal-**R**egulated **K**inase (ERK), **M**itogen-**A**ctivated **P**rotein **K**inase (MAPK)

Top 25 Hits with Sensor Peptide AQT1076 in Kinome Profiling Across 407 Kinases

AssayQuant's Kinome profiling service platform features 407 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.


AssayQuant's Kinome Tree
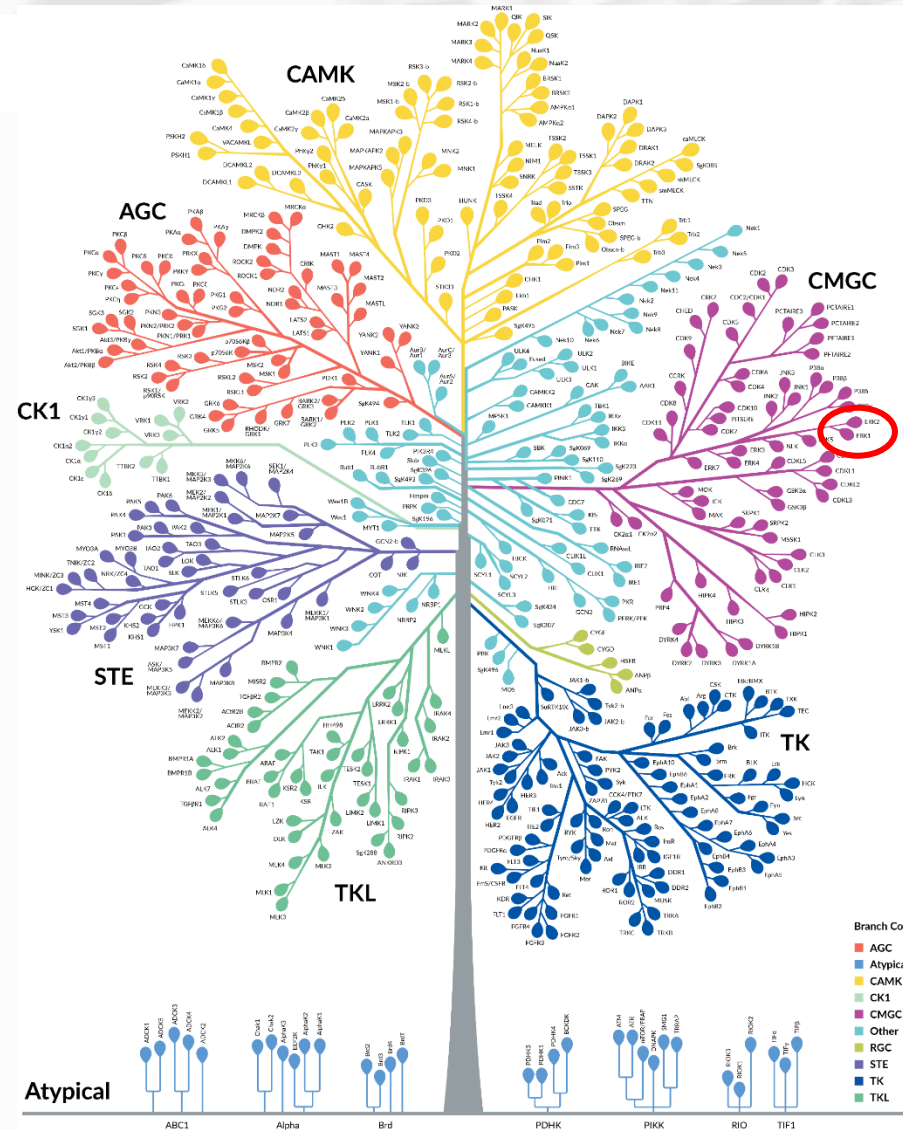
The top off-target kinase is MAPK12 or p38γ at only 1.1%.

Kinase target	Enzyme conc. (nM)	Average (RFU/pmol/min)	Rank	Selectivity Ratio	% Activity
ERK2	0.4	8,808	1	1.0	100.0
ERK1	0.4	8,109	2	1.1	92.1
P38G	0.4	93.6	3	94.1	1.1
NEK2	0.5	43.7	4	201.5	0.5
MK2	1	40.2	5	218.9	0.5
DYRK3	5	40.0	6	220.2	0.5
P38D	0.4	38.5	7	229.0	0.4
NEK9	0.5	31.0	8	284.1	0.4
SNRK	10	29.7	9	296.1	0.3
AKT3	1	25.6	10	344.0	0.3
MAP3K9	3	24.7	11	357.1	0.3
AURC	2.7	24.0	12	367.1	0.3
ERK5	5.3	23.8	13	370.1	0.3
PKACA	0.5	22.0	14	399.5	0.3
PKCQ	0.75	21.6	15	407.9	0.2
CAMK2B	0.5	20.8	16	424.3	0.2
CK1D	1	20.1	17	437.7	0.2
JNK3	5	19.7	18	446.5	0.2
PRKX	2	19.7	19	446.8	0.2
MARK2	1	19.0	20	463.2	0.2
DCAML1	3.6	17.0	21	517.1	0.2
AMPK223	2	16.2	22	543.3	0.2
AMPK213	1	15.2	23	579.1	0.2
CDK2/A2	2	14.5	24	606.2	0.2
MAP3K11	2.5	14.0	25	631.1	0.2

Selectivity of AQT1076 via AQT's Kinome Profiling

Top 25 kinases from the profiling run of 407 kinases
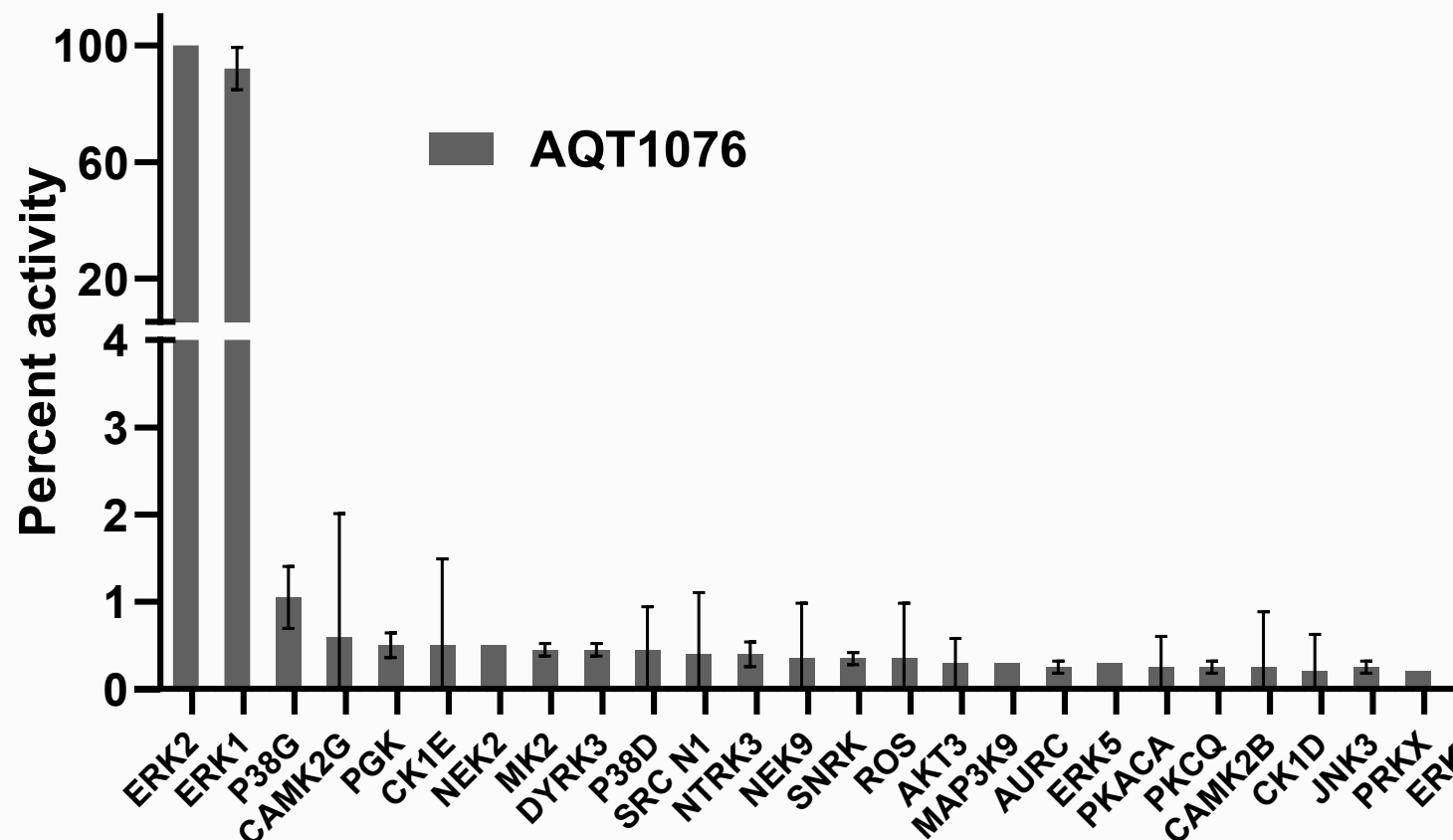
High-throughput Milestone (M1-M4) Optimization led to the identification of the AQT1076 sensor peptide substrate.

The top 25 off-target kinases were reassessed and showed *only 1.1% or less of the ERK2 signal.*

AQT1076 shows exquisite selectivity for the target kinases, a critical requirement for accurately measuring the target kinase activity in crude lysates

Outline for this Study

PhosphoSens–Lysate Assay Validation

Lysate Source:

NIH-3T3 cells +/- 25 ng/mL PDGF-bb

See slide 8 for Preparation of Crude Cell Lysates from NIH3T3 Cells Treated +/- PDGF to Show Activation of ERK1/2

Reference Compound Information:

SCH772984

Vx-11e

Experimental Validation at AssayQuant:

NIH-3T3 cell lysate (+/- 25 ng/mL PDGF to show ERK1/2 activation) and lysate (+PDGF) titration

Phosphopeptide Control (AQT1107)

Western Blotting Detection

Detecting ERK1/2 Activity Across a Variety of Cell Types

AQT1076 substrate K_m determination

DMSO Tolerance Test

Reference Compound IC_{50} Determinations with SCH772984 and Vx-11e

Preparation of Crude Cell Lysates from NIH3T3 Cells Treated +/– PDGF for Activation of ERK1/2

1) NIH-3T3 Cells were plated in 6-well tissue culture-treated plates and incubated for 48 hours at 37°C in DMEM Medium with 10% FBS and PenStrep in an atmosphere of 5% CO_2. Cells were then serum-starved in culture medium with 0.1% FBS for 24 hours and incubated for 15 minutes with or without 25 ng/mL PDGF-bb. Cells were then washed with PBS, and lysed with lysis buffer containing:

- 50 mM HEPES, pH 7.4
- 150 mM NaCl
- 2 mM EGTA
- 1 mM DTT
- 1% Triton X-100
- 30 mM NaF
- 10 mM $Na_4P_2O_7$

- 100 µM Na_3VO_4
- 50 mM β-glycerophosphate
- PhosphoPreserve Protease Inhibitor Cocktail (AQT60XPTIC)
- PhosphoPreserve Phosphatase Inhibitor Cocktail (AQT60XPPIC)

2) The DNA strands were broken by briefly sonicating on ice for 2 seconds on low power. Lysates were used immediately or aliquoted and frozen at –80 °C. 1 µg of each Lysate was run on a gel, transferred to a nitrocellulose membrane, and the signal developed by Western blotting using antibodies to total ERK1/2 and phospho-ERK1/2 (T202/Y204).

Basal vs Stimulated and Lysate Titration

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 AQT1076

Crude Lysate in µg/well of total protein (determined by the Bradford method) from NIH-3T3 cells activated treated +/- 25 ng/mL PDGF:

- Single dose: 0.25 µg/well
- Titration: 0, 0.039, 0.078, 0.16, 0.31, 0.63, 1.3, 2.5, 5, 10 µg/well NIH3T3 lysates (from cells activated with 25 ng/mL PDGF)

Reaction Set Up:

20 µL Reaction Mix with AQT1076, ATP, & DTT
15 minutes incubation at 30°C (in the reader)
5 µL Lysate (diluted to 5x in Enzyme dilution buffer, EDB) 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 round 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.
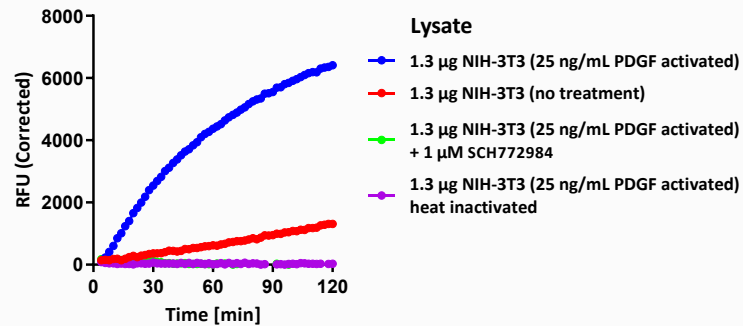
Notes:

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.
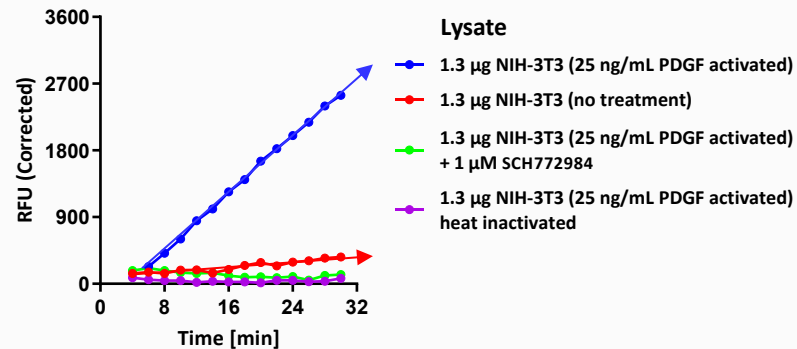
ERK1/2 Lysate Activity Assay

To Assess Activation of ERK1/2 in Lysates from + PDGF-treated Cells
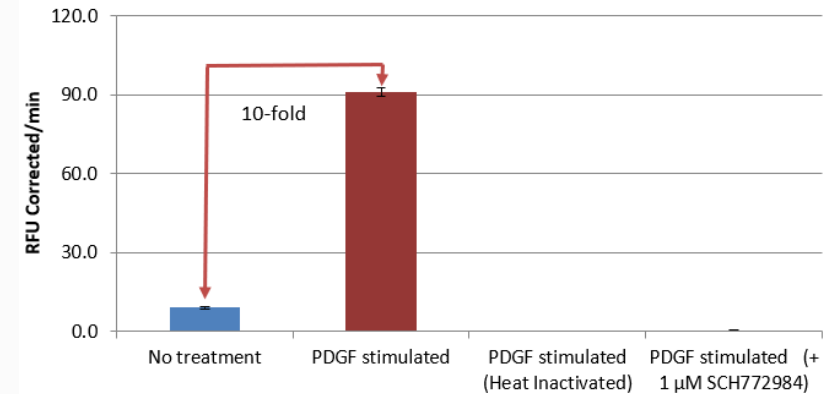
A. Crude Lysate Samples
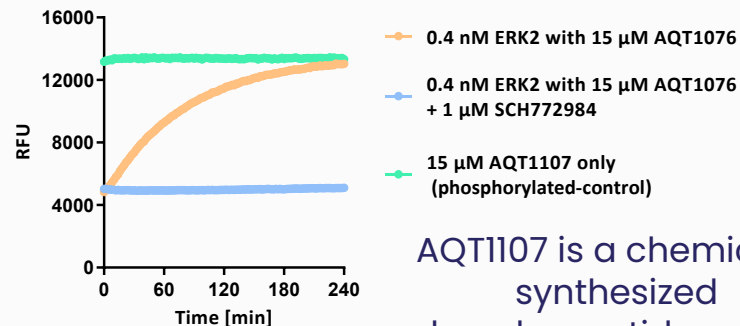
1) Full Time Course (0-120 min.)

Lysate
- 1.3 µg NIH-3T3 (25 ng/mL PDGF activated)
- 1.3 µg NIH-3T3 (no treatment)
- 1.3 µg NIH-3T3 (25 ng/mL PDGF activated) + 1 µM SCH772984
- 1.3 µg NIH-3T3 (25 ng/mL PDGF activated) heat inactivated

2) Linear Range (4-30 min.)

Lysate
- 1.3 µg NIH-3T3 (25 ng/mL PDGF activated)
- 1.3 µg NIH-3T3 (no treatment)
- 1.3 µg NIH-3T3 (25 ng/mL PDGF activated) + 1 µM SCH772984
- 1.3 µg NIH-3T3 (25 ng/mL PDGF activated) heat inactivated

3) Histogram

10-fold

B. Purified ERK2 & AQT1107 Control
1) Full Time Course (0-240 min.)

- 0.4 nM ERK2 with 15 µM AQT1076
- 0.4 nM ERK2 with 15 µM AQT1076 + 1 µM SCH772984
- 15 µM AQT1107 only (phosphorylated-control)

AQT1107 is a chemically synthesized phosphopeptide control

A. Crude lysate samples: The AQT1076 sensor peptide was used to generate RFU Corrected values (Total – Background) for **1)** Full progress curve time course (0-120 min.), and **2)** Linear range (4-30 min.), which was used to determine the slope for each condition and the results are shown as Reaction rates (RFU Corrected/min. +/- standard deviations) as a histogram in **3)**, highlighting a 10-fold activation of ERK1/2 kinase activity in lysates from NIH3T3 cells treated with PDGF at 25 ng/mL for 15 minutes. The signal was eliminated by heat inactivation of the lysate or by adding the selective ERK1/2 inhibitor SCH772984. 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 nature, concentration, and duration of the activating stimulus. These conditions can be varied to determine the effect on ERK1/2 activity. The total amount of ERK1 and 2 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 ERK2 enzyme & AQT1107 Control: The ERK2 protein (0.4 nM) fully phosphorylated the AQT1076 sensor peptide substrate by 240 min., as shown by convergence with the signal obtained with the AQT1107 phosphopeptide positive control (a flat horizontal line defining the maximum RFU with this sensor peptide). The signal with ERK2 enzyme was eliminated by adding the SCH772984 inhibitor. The signal with AQT1107 is used to convert RFU (Corrected) values to nmoles of Phosphate.
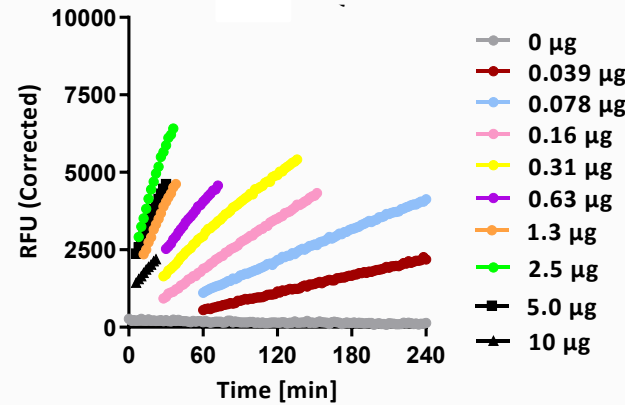
Lysate Titration

To Determine the Linearity and Sensitivity Using Lysate from + PDGF-treated Cells

AssayQuant®
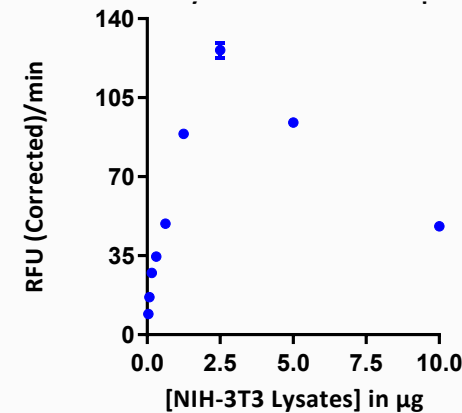TECHNOLOGIES INC.

1. Full Time Course Progress Curves
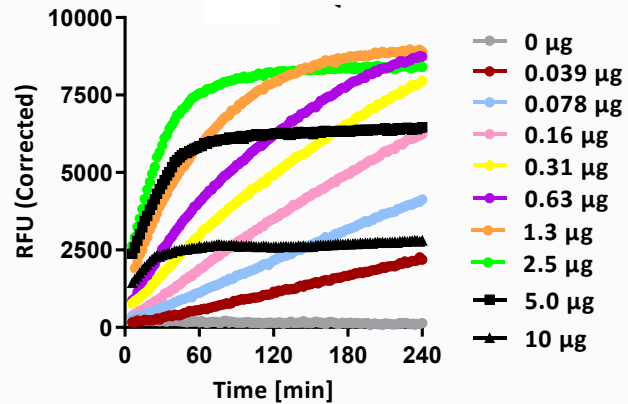
2. Linear Range of Progress Curves

3. Reaction Rate vs Amount of Lysate per Well

A. All Lysate Amounts

B. Lysate Amounts in Linear Range

Legend (charts 1 & 2):
- 0 µg
- 0.039 µg
- 0.078 µg
- 0.16 µg
- 0.31 µg
- 0.63 µg
- 1.3 µg
- 2.5 µg
- 5.0 µg
- 10 µg

Chart 1 axes: RFU (Corrected) vs Time [min], 0–240, RFU 0–10000

Chart 2 axes: RFU (Corrected) vs Time [min], 0–240, RFU 0–10000

Chart 3A axes: RFU (Corrected)/min (0–140) vs [NIH-3T3 Lysates] in µg (0.0–10.0)

Chart 3B: $r^2 = 0.9714$; axes RFU (Corrected)/min (0–140) vs [NIH-3T3 Lysates] in µg (0.0–2.5)

**Linear range (78–2,500 ng, 32-fold)**
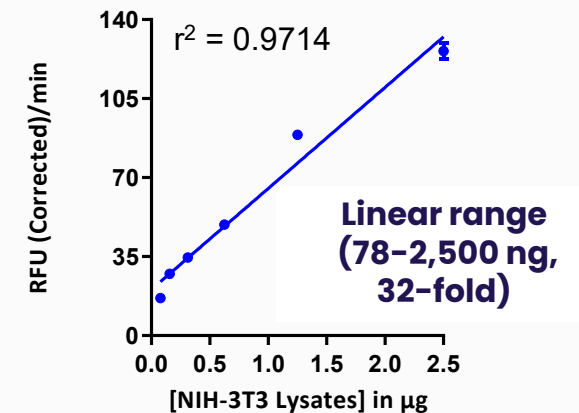
The AQT1076 sensor peptide was used at 15 µM with an increasing amount of lysate from NIH3T3 cells treated with PDGF to activate the ERK1/2 MAPK enzymes. RFU Corrected values (Total – Background) were determined for each condition. The results are presented for each amount of lysate for **1)** Full time course of each progress curve (0-240 min.), and **2)** Linear range of each progress curve, which was used to determine the slope for each amount of lysate. The results were then plotted as Reaction rates (RFU Corrected/min. +/- standard deviations) for all lysate amounts **3A)**, or those within the linear range as determined by an r^2 value > 0.95. Having the concentration of crude lysate samples at 1 mg/mL or higher, ensures that the amount of CEB in the reaction is minimized, even at the highest concentrations to avoid any inhibition of the kinase activity that can reduce the linear range.

The continuous format allows assessment of initial reaction rates from the linear region of each progress curve for an accurate, precise, and quantitative assessment of ERK1/2 activity
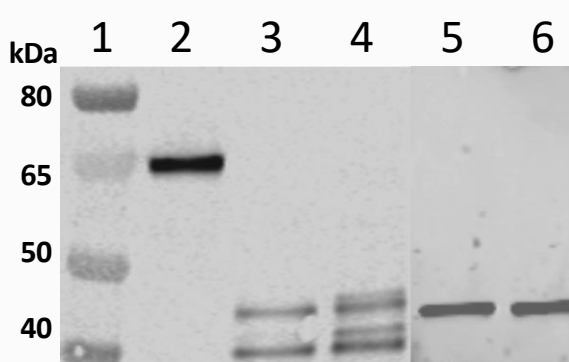
ERK1/2 Lysate Western Blots
A Commonly Used Method to Assess ERK1/2 Activation

Phospho-ERK1/2 pTEpY
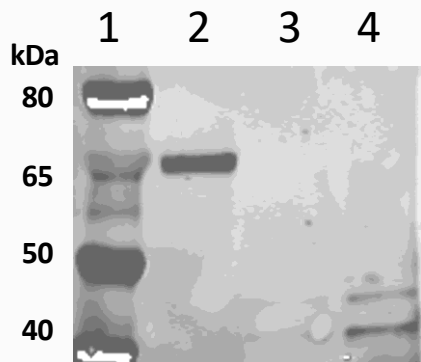
Loading Controls
Total ERK1/2   Actin

Lane Description:

1. MW Markers
2. Recombinant ERK2 (8 ng)
3. NIH-3T3 (no activation)
4. NIH-3T3 (+ 25 ng/mL PDGF for 15 minutes)
5. NIH-3T3 (no activation) – Loading Control
6. NIH-3T3 (+ 25 ng/mL PDGF for 15 minutes) – Loading Control

Method: Western blots were developed with antibodies from Cell Signaling Technology for total ERK1/2 (4696), dual-phospho-ERK1/2 (4370), or anti-beta actin (4970) and then LI-COR, IR Dye goat anti-mouse (800CW 926-32210) or donkey anti-rabbit antibody (680RD 926-68073) antibodies, followed by imaging in a LI-COR Odyssey.

Shows PDGF-induced phosphorylation of the pTEpY site in the activation loop of ERK1/2, while total ERK1/2 levels remain the same.

The MAPK family enzymes undergo dual phosphorylation in the activation loop of ERK1/2/5 (pTEpY), p38$\alpha/\beta/\gamma/\delta$ (pTGpY), and JNK1–3 (pTPpY) isoforms. Phosphospecific antibody recognition of this pTXpY motif is commonly used as a surrogate measure of activity. However, each kinase has at least 20 validated phosphorylation sites that can regulate kinase activity negatively or positively. Recent reports have also emphasized that mutations distant from the catalytic site may have long-distance effects on enzyme functions and stability and that dual phosphorylation of the pTXpY motif is not always required for activity or oncogenicity; see Petrosino *et al.,* 2023, *Human Genomics* 17:95, Cancer-related missense mutations on … properties of MAPK1 and MAPK3 somatic variants, and Soudah *et al.,* 2023, *J. Biol. Chem.* 299(9) 105072, ERK1/2 …autoactivation and oncogenic capabilities.

The solution: *Measure ERK1/2 activity directly using the PhosphoSens-Lysate assay!*

ERK1/2 Lysate Activity Assay Using the AQT1076 Sensor Peptide Across a Variety of Cell Types

❖ AQT1076 was used to assay ERK1/2 activity in lysates (1 -5 µg of total protein per 384-well) from seven cell lines. Reaction rates (RFU Corrected/pg of total lysate protein/min) were determined from the slopes using the linear portion of each progress curve. Values are the average of duplicate reactions +/- standard deviation.

❖ Normal NIH/3T3 cells could be made quiescent with serum deprivation, followed by induction with growth factor (PDGF) stimulation. In contrast, ERK1/2 was constitutively active in the other cell lines with no change with growth factor stimulation (data not shown).

❖ A tenfold increase in the rate of AQT1076 phosphorylation over the basal activity was observed with PDGF-stimulated NIH/3T3 cells (Green bar).

❖ Incorporation of the ERK1/2-selective inhibitor, SCH77984 @ 1 µM, blocked the signal with AQT1076, highlighting the selectivity of the sensor peptide for evaluating ERK1/2 activity in these complex samples.

AQT1076 (15 µM) Sensor Peptide Activity in Lysates from 7 Cell Lines ± PDGF (NIH/3T3 only) and ERK1/2 Inhibitor
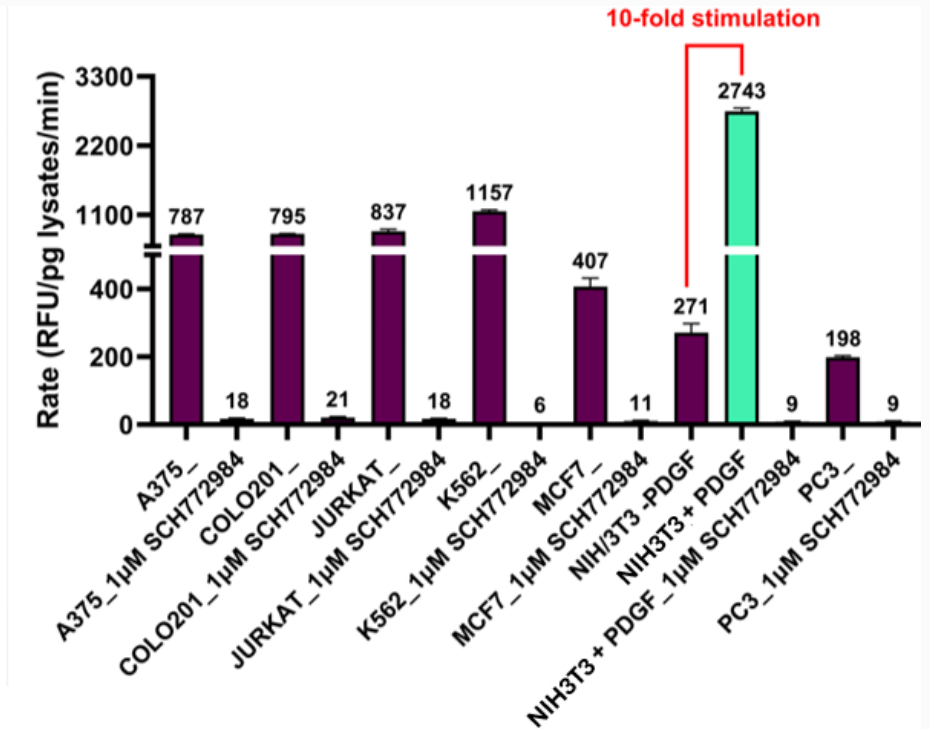
AQT1076 enables selective and precise quantitation of ERK1/2 activity with different cell types, providing a powerful tool for evaluating pathway activation and inhibition in complex samples from normal and disease states

Detecting ERK1/2 Activity with AQT1076 Across a Variety of Cell Types

Additional information on cell lines and data values

No	Cell line	Origin	Normal or Disease	Tissue	Morphology	Growth properties
1	NIH/3T3	Mouse	Normal	Embryo	Fibroblast	Adherent
2	COLO201	Human	Adenocarcinoma; Colorectal; Dukes' type D	Colon	Slightly refractile, bipolar, fibroblast-like	Suspension with some adherent cells
3	A375	Human	Malignant Melanoma	Skin	Epithelial	Adherent
4	K562-r	Human	Chronic Myeloid Leukemia At Blast Crisis	Bone; Marrow	Hematopoietic	Suspension
5	MCF7	Human	Adenocarcinoma	Breast; Mammary gland	Epithelial	Adherent and/or suspension
6	PC3	Human	Adenocarcinoma; Grade IV	Prostate	Epithelial	Adherent
7	JURKAT	Human	Acute T cell leukemia	Peripheral blood	Lymphoblast	Suspension

#	Cell line lysate	Lysate Conc. (µg)	Rate (RFU/min)	Rate (RFU/min), SE	Rate (RFU/pg lysate/min)	Rate (RFU/pg lysate/min), SE	% Inhibition by SCH772984
1	NIH/3T3-stimulated_+ PDGF	1.3	89.1	1.7	2,742.8	52.3	
2	NIH/3T3_- PDGF (Untreated)	1.3	8.8	0.9	270.8	26.8	
3	NIH/3T3 _+ PDGF_+ 1µM SCH772984	1.3	0.3	0.0	8.6	1.2	**96.8**
4	COLO201_Untreated	1	19.9	0.1	794.8	5.3	
5	COLO201_Untreated_+ 1µM SCH772984	1	0.5	0.1	20.9	2.3	**97.4**
6	A375_Untreated	1	19.7	0.2	786.8	8.4	
7	A375_Untreated_+ 1µM SCH772984	1	0.4	0.0	17.7	1.4	**97.7**
8	K562_Untreated	5	144.6	2.5	1,156.8	19.9	
9	K562_Untreated_+ 1µM SCH772984	5	0.8	0.0	6.4	0.2	**99.4**
10	MCF7_Untreated	5	50.9	3.1	406.9	24.5	
11	MCF7_Untreated_+ 1µM SCH772984	5	1.4	0.2	11.0	1.6	**97.3**
12	PC3_Untreated	5	24.8	0.6	198.1	4.4	
13	PC3_Untreated_+ 1µM SCH772984	5	1.1	0.3	8.7	2.1	**95.6**
14	JURKAT_Untreated	5	104.6	3.8	836.8	30.8	
15	JURKAT_Untreated_+ 1µM SCH772984	5	2.2	0.1	17.9	0.7	**97.9**

AQT1076 enables selective and precise quantitation of ERK1/2 activity with different cell types, providing a powerful tool for evaluating pathway activation in complex samples from normal and disease states

ERK1/2 Sensor Peptide K_m Determination

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 $MgCl_2$

0, 0.20, 0.39, 0.78, 1.6, 3.1, 6.3, 13, 25, 50, and 100 µM AQT1076

1.0 µg/well NIH-3T3 crude cell lysate (from cells activated with 25 ng/mL PDGF)

Reaction Set Up:

5 µl 5X AQT1076 Substrate dilutions
15 µL Reaction Mix with ATP & DTT
15 minutes incubation at 30 °C (in the reader)
5 µL Lysate (diluted to 5x in Enzyme dilution buffer, EDB) 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 round 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.
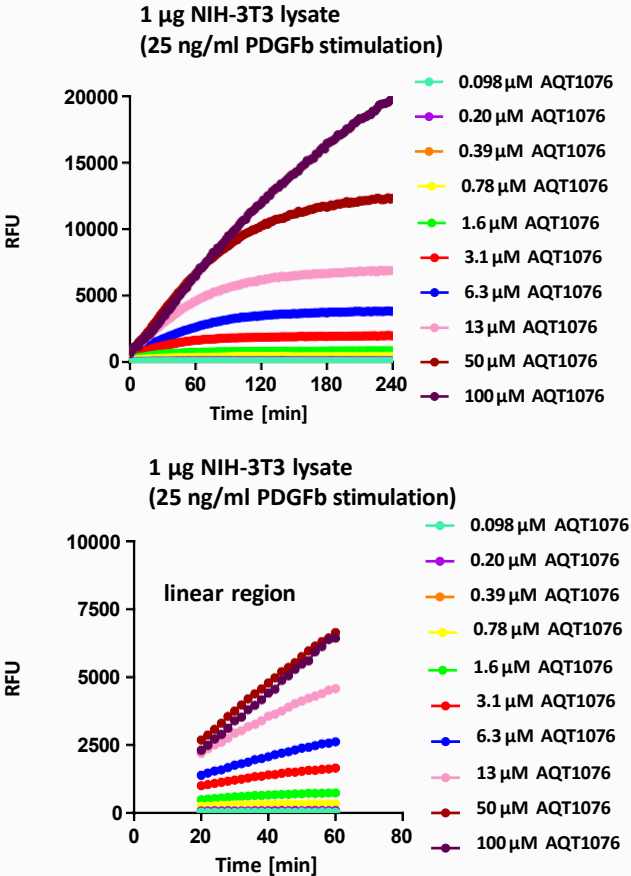
Notes:

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.
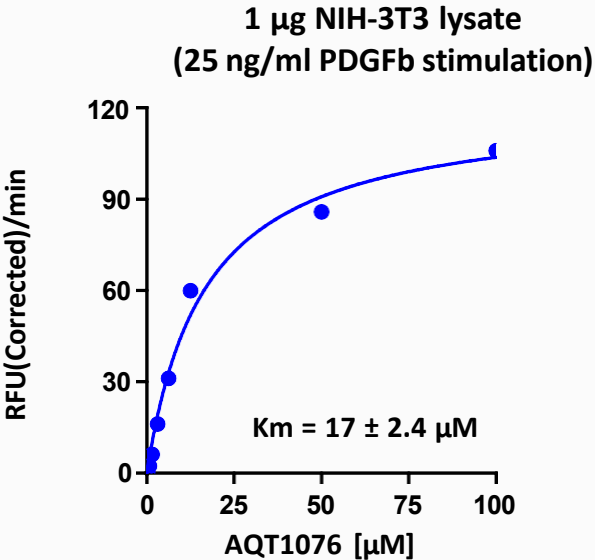
Sensor Peptide K$_m$ Determination

Titration Curves, K$_m$ Plot and Tables Using Lysate from + PDGF-treated Cells

Sensor Peptide Titration Curves

1 µg NIH-3T3 lysate
(25 ng/ml PDGFb stimulation)

- 0.098 µM AQT1076
- 0.20 µM AQT1076
- 0.39 µM AQT1076
- 0.78 µM AQT1076
- 1.6 µM AQT1076
- 3.1 µM AQT1076
- 6.3 µM AQT1076
- 13 µM AQT1076
- 50 µM AQT1076
- 100 µM AQT1076

1 µg NIH-3T3 lysate
(25 ng/ml PDGFb stimulation)

linear region

Sensor Peptide K$_m$ Plot

1 µg NIH-3T3 lysate
(25 ng/ml PDGFb stimulation)

Km = 17 ± 2.4 µM

Sensor Peptide K$_m$ Table

Michaelis-Menten Best-fit values	
Vmax	121.0
Km	16.65
Std. Error	
Vmax	5.875
Km	2.430
95% CI (asymptotic)	
Vmax	107.5 to 134.6
Km	11.04 to 22.25
Goodness of Fit	
Degrees of Freedom	8
R squared	0.9898
Sum of Squares	142.2
Sy.x	4.216

	AQT1076 K$_m$ (µM)
NIH-3T3 (25 ng/mL stimulated) Lysate	17
Recombinant FL tagless ERK1 (Sino, M29-10U)	9.3
Recombinant FL GST-ERK1 (Sino, M29-10G)	9.3
Recombinant FL GST-ERK2 (Sino, M28-10G)	14

The K$_m$ value for AQT1076 is 17 µM, similar to the K$_m$ values observed for recombinant ERK1 and ERK2

DMSO Tolerance Test

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 AQT1076

1.0 µg/well NIH-3T3 crude cell lysate (from cells activated with 25 ng/mL PDGF)

0-10% DMSO

Reaction Set Up:

2.5 µL 10X DMSO Titration

17.5 µL Reaction Mix with CSx Substrate, ATP & DTT

15 minutes incubation at 30 °C (in the reader)

5 µL Lysate (diluted to 5x in Enzyme dilution buffer, EDB) 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 round 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.
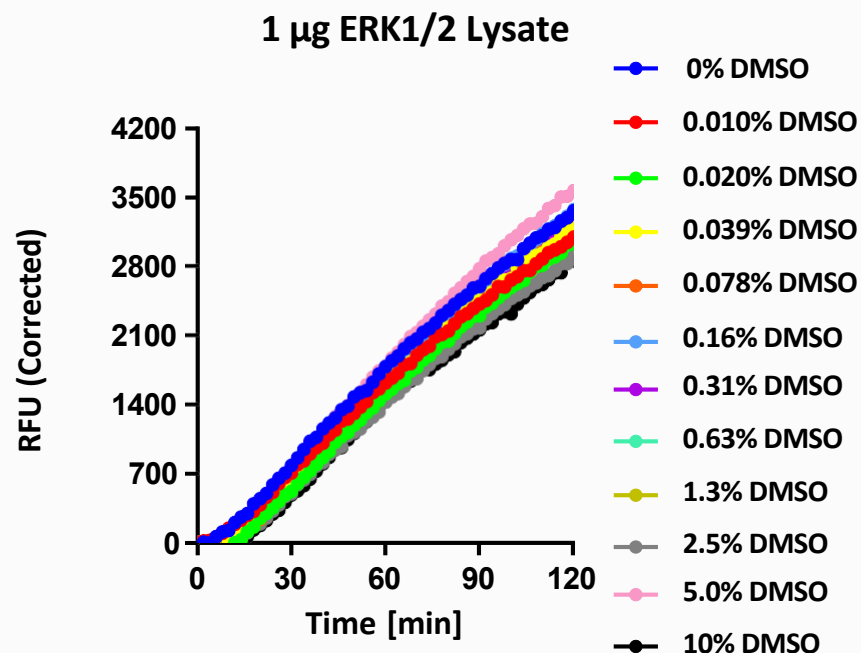
Notes:

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.
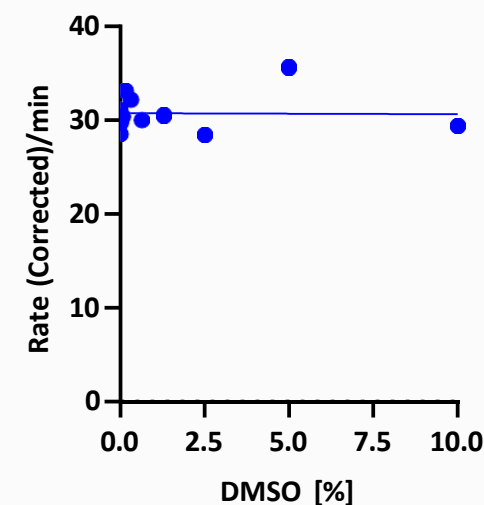
DMSO Tolerance Test

Titration Curves and Inhibition Plot Using Lysate from + PDGF-treated Cells

Complete Progress Curves

Reaction Rate vs [DMSO] Plot

No change in enzyme activity out to 10% DMSO

IC$_{50}$ Determination

Reaction Conditions and Set Up

Reaction Conditions:

54 mM HEPES, pH 7.5

1.0 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 AQT1076

2% DMSO

0-1.0 µM SCH772984

0-1.0 µM Vx-11e

1.0 µg/well NIH-3T3 crude cell lysate (from cells activated with 25 ng/mL PDGF)

Reaction Set Up:

0.5 µL 50X Inhibitor dilutions in 100% DMSO

19.5 µL Reaction Mix with CSx Substrate, ATP & DTT

15 minutes incubation at 30°C (in the reader)

5 µL Lysate (diluted to 5x in Enzyme dilution buffer, EDB) 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 round 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.
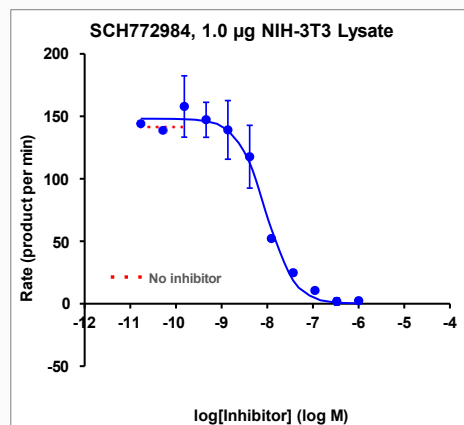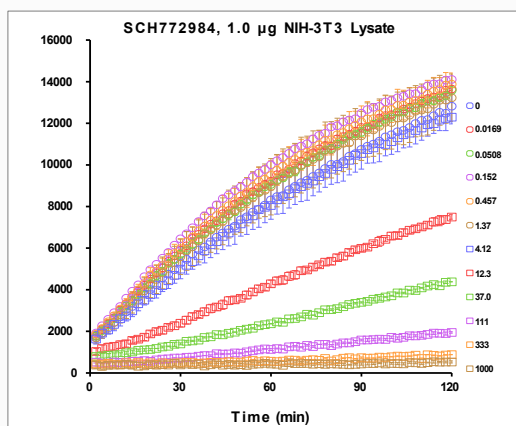
IC$_{50}$ Determination

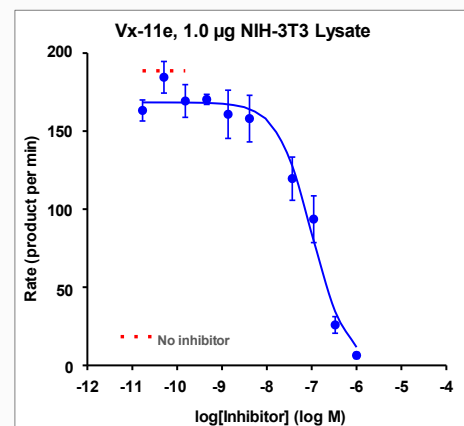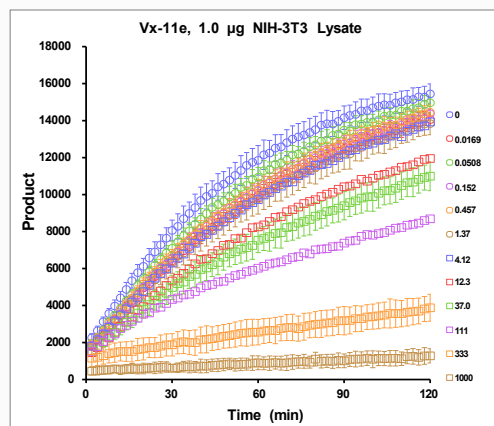Assess Compound Potency Using Lysate from + PDGF-treated Cells

AssayQuant® TECHNOLOGIES INC.

Progress Curves

IC$_{50}$ Curve

SCH772984

SCH772984, 1.0 µg NIH-3T3 Lysate

SCH772984, 1.0 µg NIH-3T3 Lysate

SCH772984 IC$_{50}$ value is 9.3 nM

Vx-11e

Vx-11e, 1.0 µg NIH-3T3 Lysate

Vx-11e, 1.0 µg NIH-3T3 Lysate

Vx-11e IC$_{50}$ value is 101 nM

Summary

NIH-3T3 cell activation with 25 ng/mL PDGF-bb increases ERK1/2 kinase activity 92-fold above the unstimulated control and is inhibited fully by 1 µM of both SCH772984 and Vx-11e reference compounds. This PhosphoSens-Lysate Assay for ERK1/2 enables direct and highly quantitative measurements in an easy-to-use format. Dual phosphorylation of ERK1/2 by western blotting, commonly used as a measure of activation, was also demonstrated and consistent, although this method is only semi-quantitative. Moreover, there are more than 20 phosphorylation sites on ERK1/2, with both positive and negative regulation, that need to be taken into account.

The PhosphoSens-Lysate Assay for ERK1/2 using the AQT1076 selective sensor peptide provides a robust and more physiologically relevant assay that measures endogenous ERK1/2 activity with all the cellular components and functional signaling complexes. Results demonstrated include:

- ❖ The ERK1/2 Lysate titration linearity from 0.31 to 10 µg/well (32-fold). This linear range is much wider than the 4-fold obtained with recombinant ERK1 or 2 enzymes.

- ❖ Sensor peptide substrate AQT1076 has a K_m of 17 µM.

- ❖ The IC_{50} values for SCH772984 and Vx-11e were 9.3 nM and 101 nM, respectively.