AssayQuant® TECHNOLOGIES INC.

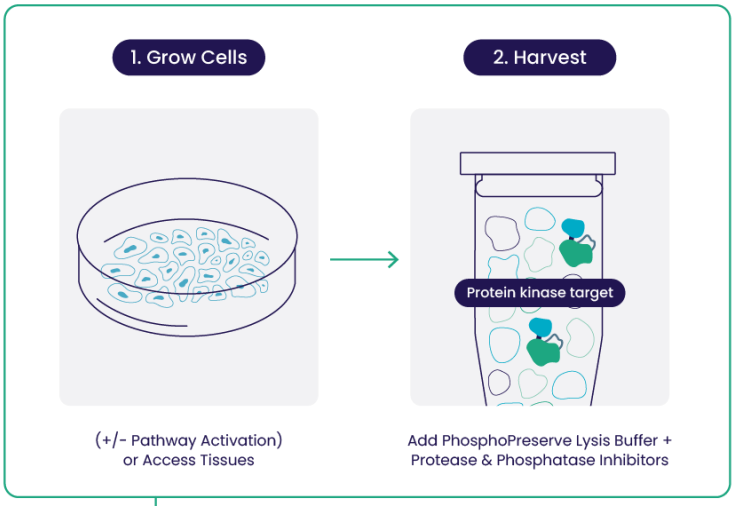
INTRODUCING CELL LYSATE KINASE ASSAYS

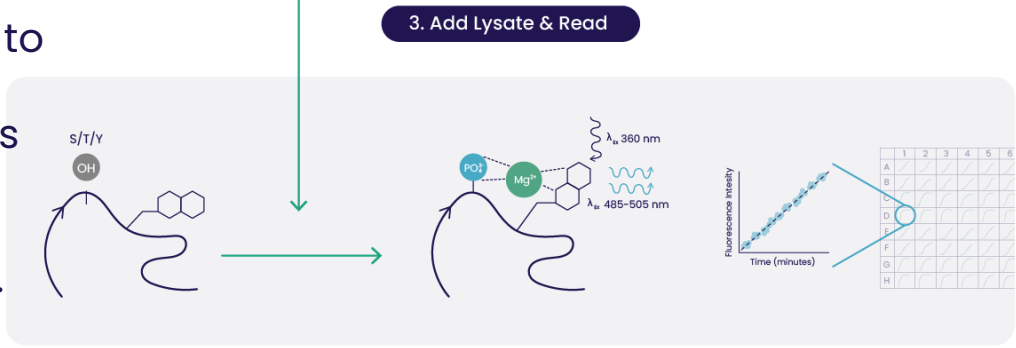
Real-Time Protein Kinase Activity Assays in Complex Biological Samples

Kinetic enzymatic assays in cell lysates and tissue homogenates – enabling precise quantitation and deeper insight into kinase and inhibitor function in a biologically relevant context.

OVERVIEW:

To address the tremendous opportunity for kinase research and drug development, we have combined innovative PhosphoSens detection technology with high-throughput peptide synthesis to design sensor peptide substrates that are highly selective for your targets of interest.





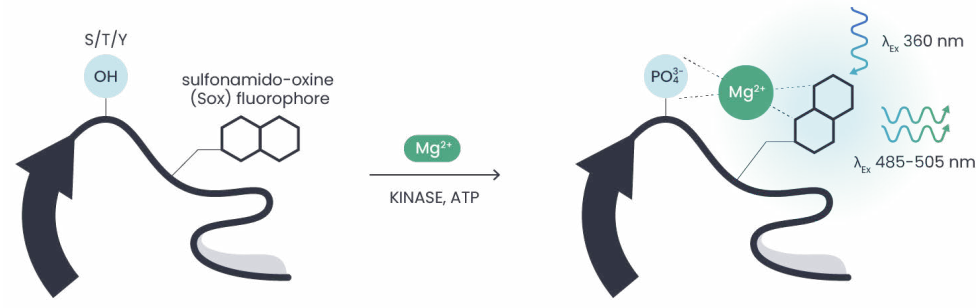
1 How the PhosphoSens Assay Works & Why This Matters

1. Selective Substrate Design:
- Each substrate is an optimized peptide selective for the kinase target.
- Proximal to the Ser/Thr/Tyr phosphorylation site, the peptide is modified with our readout molecule, Sulfonamido-oxine (Sox).

2. Activity-based Chelation-Enhanced Fluorescence (ChEF):
- When the kinase phosphorylates the peptide substrate, the new phosphate group interacts with Sox and magnesium ions (Mg^{2+}), resulting in an immediate increase in fluorescence.

3. Real-Time/Continuous Readout:
- As the kinase reaction proceeds, the fluorescence increases in direct proportion to the level of phosphorylation, generating a progress curve in ever well, enabling real-time monitoring of enzyme kinetics.

4. Direct and Homogeneous Format:
- No antibodies. No coupled enzymes. No wash steps required.
- The assay is add-and-read, making it ideal for high-throughput screening, kinetic studies, and lead optimization, providing rich data with less effort.



PhosphoSens Sensor Peptide Substrate

Better Data, Better Decisions

A continuous format yields a true rate determined from dozens of data points within the actual linear range of a progress curve, resulting in a high-confidence measurement as compared to the assumed rate inferred from an endpoint assay, which is adversely affected by many common elements of kinase activity assays (e.g., Lag Phases, Substrate Depletion, Enzyme Instability, Time-Dependent Inhibition (TDI) and Product Inhibition).

PhosphoSens Cell Lysate activity assays deliver the accuracy of conclusions drawn from a continuous assay format that is not compromised by these complicating factors, because the format not only avoids their impact, but also enables identification, characterization, and even exploitation of these modes of action within complex samples—capabilities not possible with endpoint, biophysical, or Western methods.

2 Why Study Kinase Activity In A Complex Environment

By using unfractionated cell lysates or tissue homogenates, these assays enable precise quantitation of kinase activity in complexes in their native state across multiple biological sources—offering a functional correlate between target inhibition, signal transduction network status, and disease state. By combining the efficiency of biochemical testing with the complexity of the cellular milieu, our assays account for factors that significantly influence kinase biology and inhibitor performance *in vivo*, including:

1. Cellular Components:

- Native proteins (full-length, no tags), lipids, nucleic acids, small molecules, cofactors and binding partners that form functional complexes.

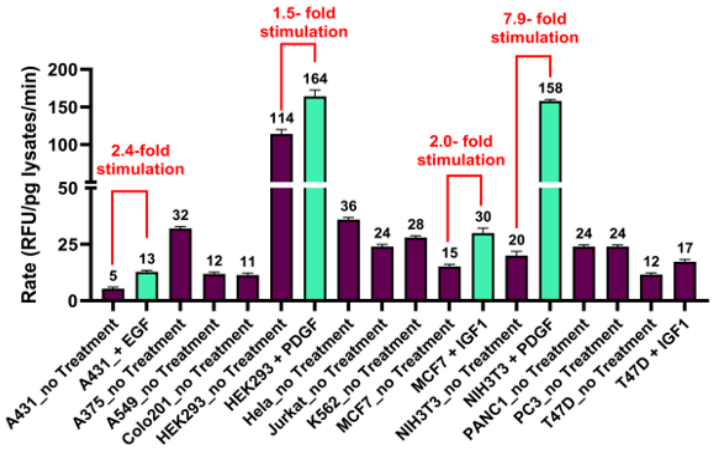
2. Post-Translational Modifications (PTMs):

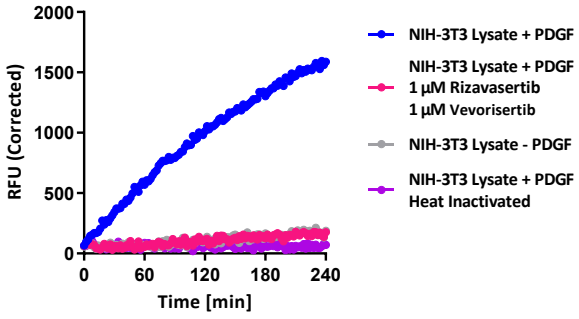
- An abundance of PTMs that can influence kinase activity, localization, and interactions, providing physiologically relevant insights.

3. Cellular Signaling Networks

- Regulatory feedback loops and signaling crosstalk that change over time during the reaction. This includes potential interfering kinases, phosphatases, and other enzymes/proteins that are important to screen against for potency and selectivity.

Using our **PhosphoSens AKT1/2/3 Cell Lysate Kinase Assay**, we measured endogenous AKT activity across a panel of cell lines under basal and stimulated conditions. The results highlight how kinase activity is dynamically modulated in a native environment—insight that cannot be obtained using recombinant enzyme alone. Furthermore, **AKT-selective inhibitors** fully blocked the signal, confirming that the response measured with AQT0982 is **target-selective and biologically relevant**.

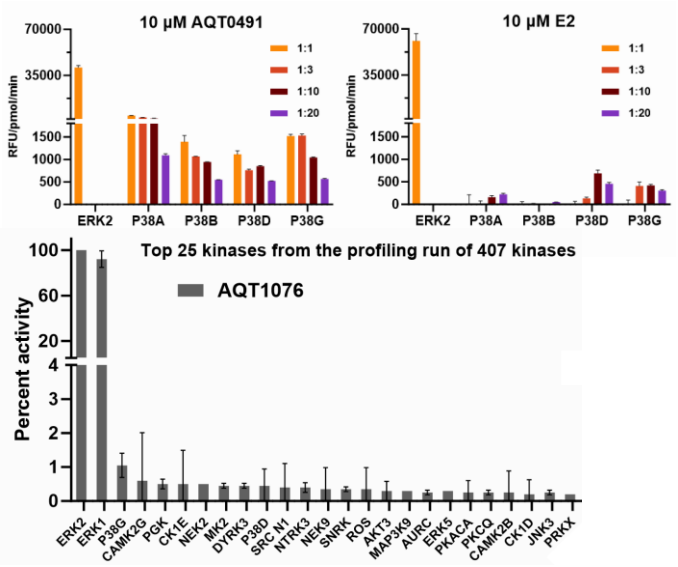




3 Rigorous Development & Validated Performance.

The ERK1/2 signaling pathway is a critical component of the MAPK cascade, regulating essential cellular processes such as proliferation, differentiation, and survival. Dysregulation of ERK1/2 activity is implicated in various diseases, including cancer, neurodegenerative disorders, and inflammatory conditions, making it a key target for drug development.

To enable ERK1/2 activity detection in a complex sample, our **milestone-based assay development approach** was initiated to design a sensor that detects the intended target without interference—ensuring accurate, meaningful data in complex sample types.



1. Milestone 1:

- Screened123 candidate sensor peptides from native substrates.
- Identified AQT0491, with high activity but moderate selectivity.

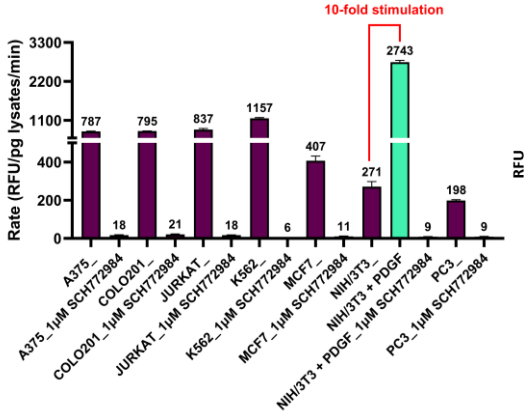
2. Milestone 2:

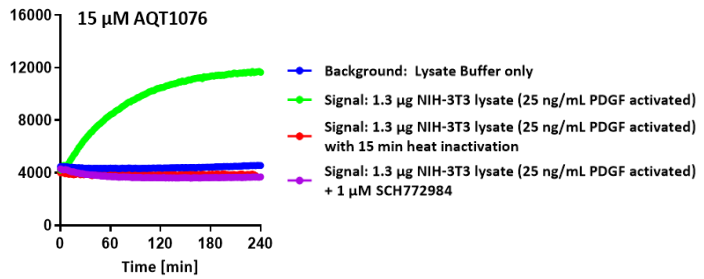
- Screened 259 sequences for ERK1/2 activity and selectivity.
- Selected optimized single amino acid substitutions that enhance activity and specificity.

3. Milestone 3:

- Combined substitutions to generate 194 peptide variants.
- Top-performing peptide (E2) showed ~10x better selectivity vs. off-target kinases and ~1.5x higher ERK2 activity than AQT0491.

The optimized **Target Selective Sensor Peptide Substrate, AQT1076**, can now be used to assay ERK1/2 in a complex sample.





AssayQuant TECHNOLOGIES INC.

Quantify Endogenous Kinase Activity and Signaling in Cell Lysates and Tissue Homogenates – No Antibodies, No Washes, Just a Continuous Signal

Search Below to Match Your Target With Kinase-Selective Lysate Substrate

Flexible Configurations Built Around Our Substrates For Your Applications:

Cell Lysate Assay Kit (AQTxxxx-KL-100):
Each kit includes sufficient volumes of substrate, reaction reagents, cell extraction reagents and controls to perform **100 reactions** at 15 µM substrate in a 384-well plate (25 µL reaction volume). Includes PhosphoSens kinase-selective lysate substrates, reaction reagents, cell extraction reagents and assay controls.

Cell Lysate Assay Kit (AQTxxxx-KL-1000):
Each kit includes sufficient volumes of substrate, reaction reagents, cell extraction reagents and controls to perform **1,000 reactions** at 15 µM substrate in a 384-well plate (25 µL reaction volume). Includes PhosphoSens kinase-selective lysate substrates, reaction reagents, and cell extraction reagents.

Kinase-Selective Lysate Substrate (AQTxxxx-L):
AssayQuant's Cell Lysate Kinase Assays combine PhosphoSens Detection Technology with high-throughput peptide synthesis to design sensor peptide substrates that are highly selective for your targets of interest. Each Kinase-Selective Lysate Substrate can be purchased as lyophilized 1mg aliquots. Each mg is sufficient for **1,500 reactions** at 15 µM substrate in a 384-well plate (25 µL reaction volume).

Target Name	HGNC Approved Full Name	Kinase-Selective Lysate Substrate	Substrate MW (g/mol)	Assay Validation Report
ERK1/2	mitogen-activated protein kinase (MAPK3/1)	**AQT1076**	2373.0	LINK >
AKT1/2/3	AKT serine/Threonine Kinase 1/2/3	**AQT0982**	3204.7	LINK >
GSK3A/B	glycogen synthase kinase 3 alpha/beta	**AQT1211**	2005.9	LINK >

Be among the first to access these kinetic enzymatic assays in complex samples!
Reach out today to learn about our Early Adopters Program with exclusive benefits.

Additional Targets In Development!
We are actively developing Cell Lysate Kinase Assays for the following targets: AKT, MEK, PIM, JNK, P38, GSK3, CDK and more!

The Best Solutions Start with a Conversation

Talk to our team at hello@assayquant.com to get started