Measuring What Matters

A Selective ERK1/2 Activity Assay for RAS/MAPK-Pathway Drug Discovery

White Paper

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Introduction

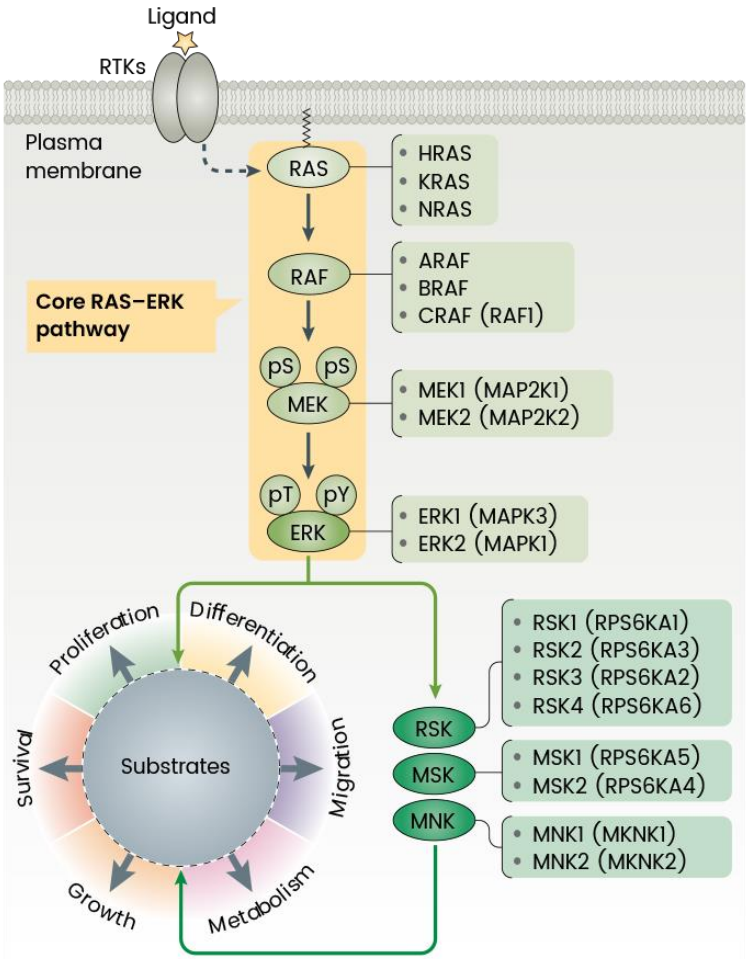
Extracellular signal-regulated kinases 1 and 2 (ERK1/2, encoded by MAPK3 and MAPK1) sit at the terminus of the RAS–RAF–MEK–ERK cascade, one of the most extensively studied and heavily drugged signaling pathways in oncology (Scheme 1). This cascade relays growth-factor and mitogenic signals to control cell proliferation, differentiation, and survival, and its dysregulation is a hallmark of a substantial fraction of human cancers, particularly those driven by RAS and RAF mutations.

Despite major advances in upstream pathway inhibitors, acquired and intrinsic resistance remain central challenges. The KRAS G12C inhibitors sotorasib and adagrasib, approved in 2021 and 2022 respectively, established a landmark precedent for direct RAS-pathway targeting — yet resistance arises routinely, often through feedback reactivation of ERK signaling via receptor tyrosine kinases or through alterations in ERK pathway components themselves. As a result, ERK1/2 has emerged both as a therapeutic target in its own right and as an essential pharmacodynamic biomarker for evaluating pathway reactivation across RAS-, RAF-, and MEK-directed regimens and their combinations.

Several direct ERK1/2 inhibitors, including ulixertinib, GDC-0994, and MK-8353, have advanced into clinical evaluation, underscoring sustained pharmaceutical interest in this node even as durable monotherapy responses remain difficult to achieve. For drug developers working anywhere along this pathway, robust and quantitative tools that measure ERK1/2 catalytic activity — not merely its phosphorylation state — are critical for compound characterization, resistance monitoring, and translational biomarker development. Here we describe the development and validation of a selective, lysate-compatible ERK1/2 activity assay suitable for both biochemical screening and cell-based pathway studies.

Sino Biological / SignalChem Biotech Perspective

While ERK1/2 serves as a critical downstream node, successful drug discovery requires high-quality reagents spanning the entire RAS/RAF/MEK/ERK signaling cascade. Researchers often investigate pathway activation, resistance mechanisms, target selectivity, and signaling cross-talk across multiple pathway components throughout the drug discovery process.
Sino Biological/SignalChem Biotech supports these efforts through an integrated platform of recombinant proteins, active kinases, phosphatases, disease-relevant mutants, antibodies, and specialized research services. From custom protein and enzyme expression to assay development and compound screening, our solutions help accelerate programs from early target validation through lead optimization and translational research.



Scheme 1: MAPK/ERK Signaling Pathway: Representative Solutions from Sino Biological/SignalChem Biotech. The distinct isoforms comprising the core RAS–ERK pathway in mammals are indicated. ERK-activated kinases of the RSK, MSK, and MNK families expand the core pathway's effectors by targeting more substrates. Figure source: https://doi.org/10.1038/s41580-020-0255-7

Development of a Selective ERK1/2 Activity Assay

The assay is built on AssayQuant's PhosphoSens® technology, a proprietary, homogeneous, fluorescence-based platform for real-time, continuous monitoring of kinase and phosphatase activity. Sensor peptide substrates are chemically synthesized with a site-specific, chelation-enhanced fluorescence (ChEF) probe that reports phosphorylation directly and continuously — with no antibodies, no radioactivity, and no wash or separation step required. Because signal is generated throughout the reaction, PhosphoSens assays capture full kinetic progress curves rather than a single endpoint, enabling accurate determination of initial reaction rates, Km values, and inhibitor potency (IC50/Ki), even in complex, crude sample matrices such as cell or tissue lysates.

PhosphoSens substrate development conditions:

- Generic substrates — optimized to report activity broadly across a kinase family or subfamily, supporting general screening and comparative profiling.

- Selective substrates — engineered through iterative peptide optimization and validated against AssayQuant's in-house profiling panel of 407 wild-type kinases.
- Assay-ready in both recombinant-enzyme and crude-lysate formats, with no antibody, radioactivity, or wash step required.
- Continuous kinetic read (up to 240 min) for accurate initial-rate, Km, and IC50/Ki determination.

As a direct application of this capability, AssayQuant developed AQT1076, a PhosphoSens sensor peptide substrate selective for ERK1/2, and screened it against AssayQuant's full panel of 407 wild-type kinases (Figure 1). ERK2 and ERK1 ranked first and second, and the top off-target kinase (MAPK12/p38γ) captured only 1.1% of the ERK2 signal — the level of selectivity required to accurately attribute signal to ERK1/2 activity even in unfractionated, complex samples.

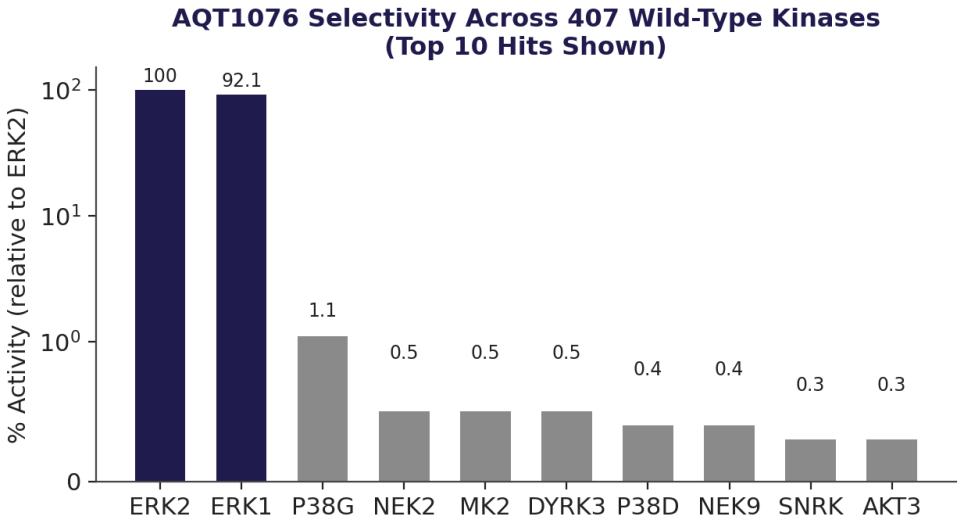


Figure 1. AQT1076 selectivity across AssayQuant's 407-kinase profiling panel (top 10 hits shown).

Kinetic Characterization Across Recombinant Enzyme Sources and Lysates

AQT1076 was characterized against recombinant ERK1 (tag-free and GST-tagged) and ERK2 (GST-tagged) enzymes sourced from Sino Biological, as well as against endogenous ERK1/2 activity in NIH-3T3 cell lysates (Table 1). Substrate and ATP Km values were consistent across all formats, confirming that the assay behaves comparably whether measuring purified Sino recombinant enzyme or native ERK1/2 activity in a crude lysate.

Enzyme Format	Source	Sensor Peptide Km (µM)	ATP Km (µM)	SCH772984 IC50 (nM)
Recombinant ERK1 (tag-free)	Sino Biological (M29-10U)	9.3	61.9	4.59
Recombinant ERK1 (GST-tag)	Sino Biological (M29-10G)	9.3	52.6	5.62
Recombinant ERK2 (GST-tag)	Sino Biological (M28-10G)	14.2	50.9	1.10
NIH-3T3 cell lysate (endogenous ERK1/2)	—	17	—	9.3

Table 1. Kinetic and potency parameters for AQT1076 across recombinant ERK1/ERK2 enzyme formats and NIH-3T3 lysate.

SCH772984 is a potent, ATP-competitive small-molecule inhibitor of ERK1/2 that binds a distinct pocket associated with the inactive kinase conformation, blocking phosphorylation of downstream substrates. It has demonstrated robust activity against tumor cells harboring BRAF, NRAS, or KRAS mutations, including models with acquired resistance to earlier BRAF and MEK inhibitors, and is widely used as a benchmark reference compound in ERK1/2 enzymology and cell-based pathway research. Because of its well-characterized potency and mechanism, SCH772984 served as the primary reference inhibitor for the IC50 determinations shown in Figure 2, both with Sino's recombinant ERK2 enzyme and in the NIH-3T3 lysate system.

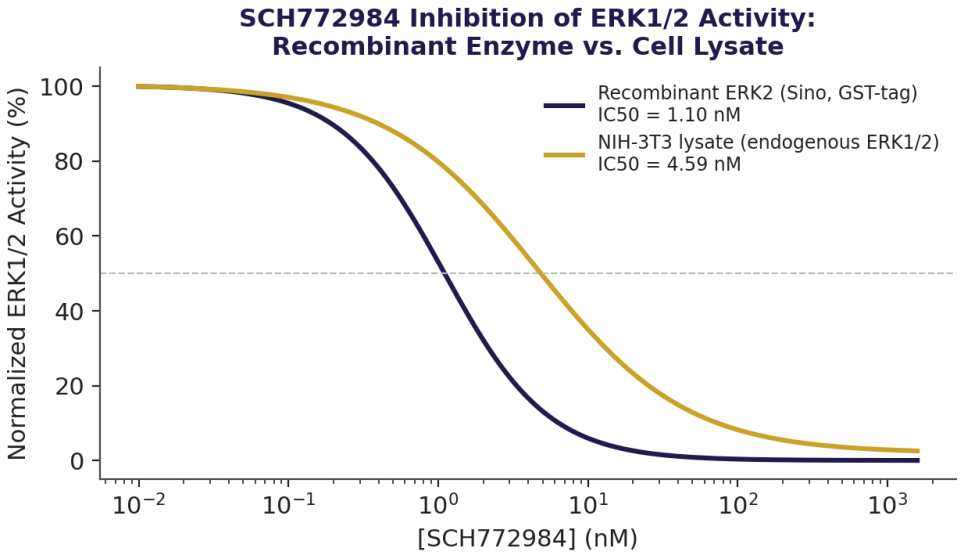
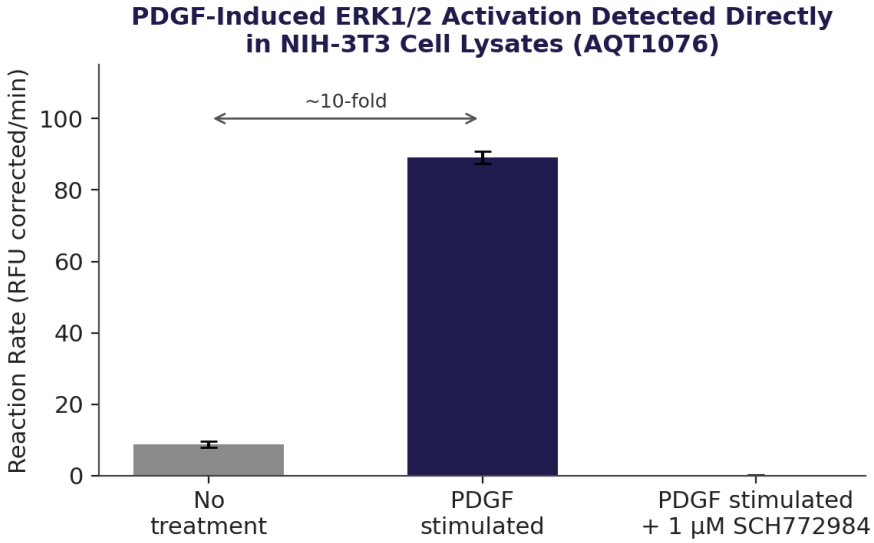


Figure 2. SCH772984 concentration-response curves for recombinant ERK2 (Sino Biological) and NIH-3T3 lysate (endogenous ERK1/2), plotted from best-fit four-parameter logistic parameters.

Detecting ERK1/2 Activity Directly in Cell and Tissue Lysates

In NIH-3T3 cells, serum starvation followed by PDGF-bb stimulation increased ERK1/2 kinase activity roughly 10-fold above the unstimulated baseline, as measured directly by AQT1076 turnover (Figure 3). This activation was fully abolished by heat-inactivating the lysate or by adding 1 µM SCH772984, confirming that the measured signal is attributable specifically to active ERK1/2. The assay's linear range spanned a 32-fold span of lysate input (0.31–10 µg/well), substantially wider than the roughly 4-fold linear range achievable with purified recombinant enzyme alone — a key advantage when working with variable, unfractionated biological samples.

Figure 3. PDGF-induced ERK1/2 activation detected directly in NIH-3T3 lysates by AQT1076, and its abolition by the ERK1/2 inhibitor SCH772984.



This direct activity read-out offers a meaningful advantage over the traditional approach of Western blotting for phospho-ERK1/2 (pTEpY), which is only semi-quantitative and captures a single regulatory mark, when ERK1/2 activity is known to be influenced by more than 20 distinct phosphorylation sites with both positive and negative regulatory effects. The PhosphoSens-Lysate format instead reports the functional output of the fully assembled, endogenous signaling complex.

AQT1076 was further used to quantify ERK1/2 activity across a panel of seven cell lines spanning normal, adenocarcinoma, leukemia, and melanoma origins (Table 2). ERK1/2 activity was readily detected in all lines and was inhibited 95–99% by 1 µM SCH772984 in every case, demonstrating that this selective, lysate-compatible substrate is broadly applicable across normal and disease-relevant cellular contexts — supporting use cases from basic pathway biology through compound profiling and translational biomarker work.

Cell Line	Origin / Tissue	SCH772984 Inhibition
NIH-3T3 (+PDGF)	Mouse embryo fibroblast	96.8%
COLO201	Human colorectal adenocarcinoma	97.4%
A375	Human melanoma	97.7%
K562	Human CML (blast crisis)	99.4%
MCF7	Human breast adenocarcinoma	97.3%
PC3	Human prostate adenocarcinoma	95.6%
JURKAT	Human acute T-cell leukemia	97.9%

Table 2. ERK1/2 activity detected by AQT1076 across a seven-cell-line panel, with inhibition by 1 µM SCH772984.

Advance Your RAS/MAPK-Pathway Drug Discovery Program

AssayQuant Technologies and Sino Biological together offer a complete toolkit for ERK1/2 and broader RAS/MAPK pathway research:

- High-purity recombinant RAS/RAF/MEK/ERK pathway kinases and phosphatases (Sino Biological)
- Selective, continuous-read PhosphoSens® activity assays for biochemical and lysate-based studies (AssayQuant)
- Custom substrate development for novel or difficult-to-assay kinase and phosphatase targets
- Combined validation packages bridging recombinant enzyme and cellular/tissue activity data

Contact us today: hello@assayquant.com | support_us@sinobiologicalus.com

Further Reading & Related Resources:
AssayQuant

Cell Lysate Kinase Assays Product Page
PhosphoSens-Lysate ERK1/2 Activity Assay Validation Report
KinSight Quantitative Kinome Profiling Services

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MAPK/Erk Signaling Pathway Map
Comprehensive Range of Active Kinases
Canada's Largest Local Protein & Enzyme CRO