Capabilities Brochure

PhosphoSens®

- **IC_{50}, EC_{50} and K_i** for catalytic inhibitor/ activator potency
- **Kinome Profiling** for compound selectivity
- **Progress Curve Analysis** for time-dependent inhibition
- **Jump Dilution** for reversible vs irreversible determinations
- **Residence Time** for reversible inhibition studies
- **k_{inact}/K_i** for irreversible inhibition studies
- **MOI/MOA** for inhibitor-substrate competition studies
- **Add Eu3+** for red-shifted endpoint HTS

Kinetic Questions Are Best Answered By A Kinetic Assay

***PhosphoSens*® technology to empower kinase and phosphatase inhibitor drug discovery.**

Role of Kinases & Phosphatases in Human Disease

Dysregulation of kinases and phosphatases has been linked to cancer, heart disease, neurodegenerative disease and more. Successful treatment relies on modulating the activity of these important enzymes with effective inhibitors. Our goal is to provide you with the best technology and services to advance your research and drug development process.

Key Components of *PhosphoSens* Technology

Proprietary Fluorophore - The Sox (sulfonamido-oxine) fluorophore is a small, minimally hydrophobic dye, which, when complexed with magnesium ion and a phosphoryl group, exhibits a fluorescence emission at 485 nm enabling continuous detection with a standard microplate reader.

Fluorogenic Sensor Peptides - Our library of over 30,000 sensor peptides substrates includes both wild type and mutant protein sequences of serine-threonine and tyrosine kinase substrates. Each peptide is covalently coupled to the Sox fluorophore at positions adjacent the site of catalysis. To date, AssayQuant has developed over 400 assays that are available in optimized kits and as standalone sensor peptides. Our extensive library along with our deep expertise enables us to provide you with a rapid turn-around for custom assay development and custom services including kinetic characterization and profiling.

Continuous	The *PhosphoSens* assay captures the entire reaction in real-time enabling determination of kinetic parameters and detailed characterization of inhibition
Direct	The Sox fluorophore detects actual phosphorylation and dephosphorylation rather than detecting a proxy such as a binding event or ADP
Catalytic	*PhosphoSens* sensor peptides are optimized to bind to the substrate binding site enabling measurement of the true and complete catalytic process
Physiologically Relevant	*PhosphoSens* sensor peptides are derived from and optimized for interaction with its target kinase or phosphatase
Versatile	The *PhosphoSens* assay is compatible with any ATP concentration - ATP K_m for profiling, and physiological ATP (1-2mM) for continuity with cell-based assays
Novel and Non-disruptive	*PhosphoSens* is novel in its delivery of rich information in every well while being non-disruptive in workflow (simple add-and-read format) and equipment (standard fluorescence microplate reader)

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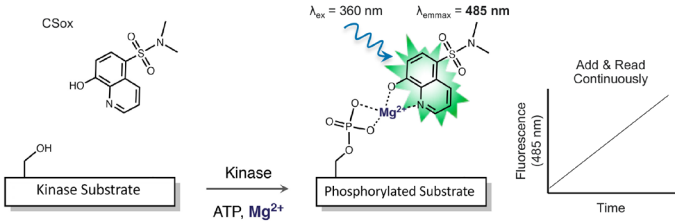
PhosphoSens Technology

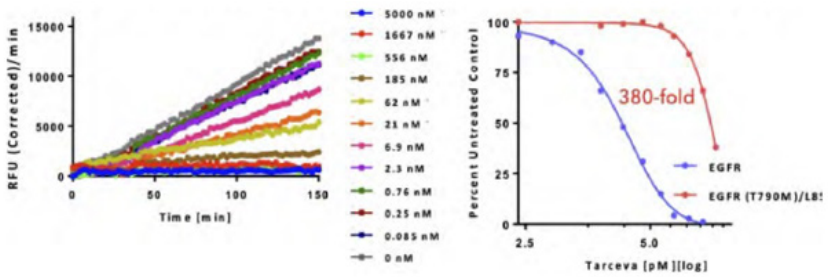
PhosphoSens®

Application Examples



IC_{50}, EC_{50} and K_i for catalytic inhibitor/activator potency

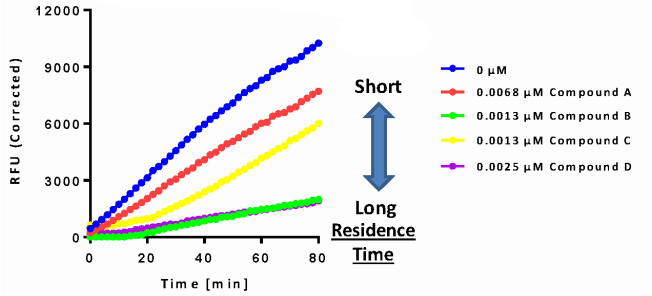
Simultaneously compare compound potency for a large number of inhibitor/activator candidates.



The EGFR inhibitor, Tarceva, is titrated (0.085nM – 5000nM) with wild type and mutant receptor. The mutant EGFR receptor, T790M/L858R, is 380-fold less sensitive to inhibition by Tarceva compared to wild type receptor, confirming drug resistance for this mutant receptor.

Jump Dilution and Residence Time for reversible vs irreversible inhibition determination

Distinguish between covalent vs. reversible inhibitors and gain rich information about time-dependence of inhibition.



Residence time (Tau) was determined using the Jump-dilution method of Copeland. Compounds are incubated with EGFR (T790M/C797S/L858R) at 25x the IC_{50} value for 60 min, followed by a 250- fold dilution and fluorescence intensity measurements revealing that Compounds B and D are irreversible and Compounds A and C are reversible.

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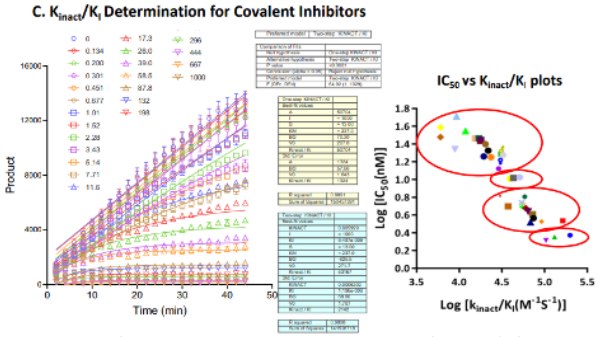
k_{inact}/K_I for irreversible inhibition studies

Use global fit analysis and a progress curve in every well to fully characterize covalent inhibitors.



k_{inact}/K_I determination showing 1-step vs. 2-step covalent inhibitor inactivation of EGFR, and a plot of Log IC vs k_{inact}/K_I illustrating the ability to differentiate compounds with very similar IC_{50} values by IC_{50} vs k_{inact}/K_I.

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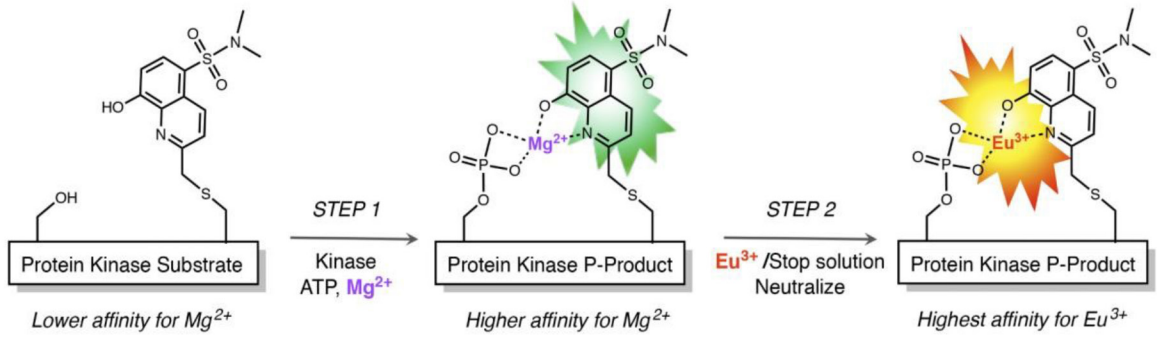
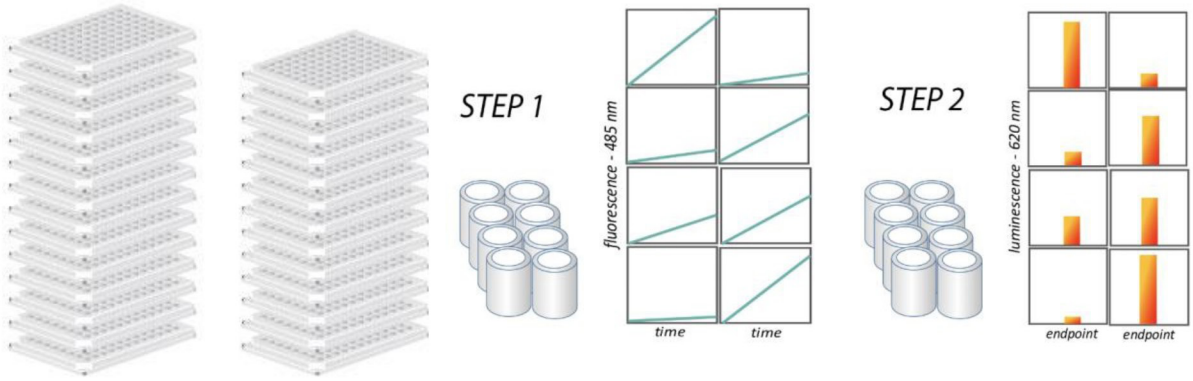
PhosphoSens - RED

Kinetic and Endpoint

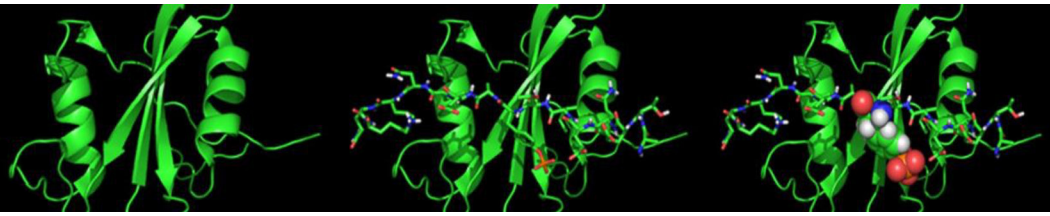
PhosphoSens provides both kinetic and endpoint readouts. Simply add Eu^{3+} to the kinetic assay to convert it to a Red-shifted endpoint assay.

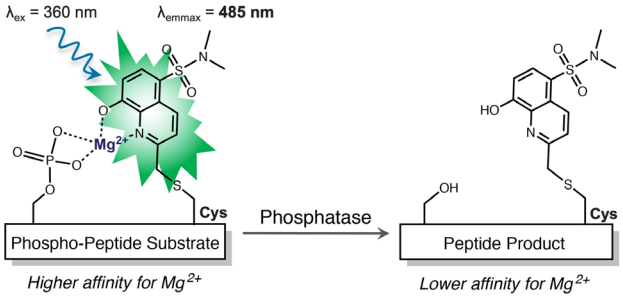
For HTS, endpoint analysis exploits direct sensitization of europium to enable detection of luminescence at 620 nm using time-resolved data after 200 µsec delay - eliminating background fluoresence.

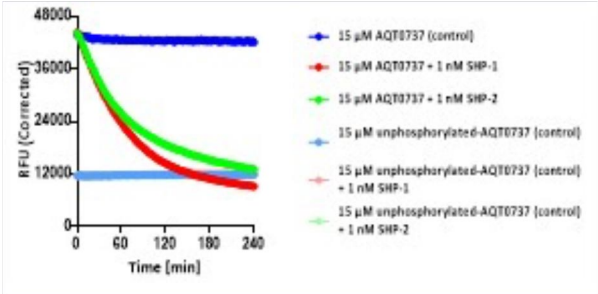




Phosphatase

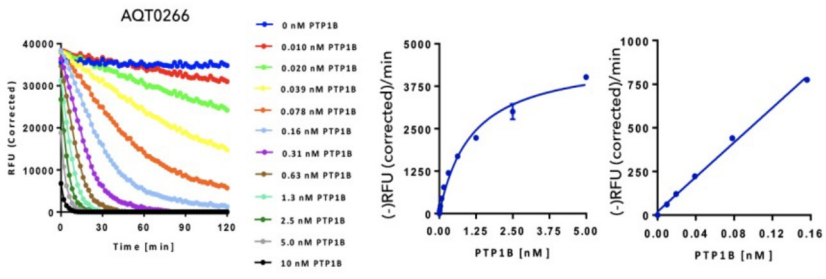






Above: *PhosphoSens* data for SHP-1 and SHP-2 with AQT0737

Right: *PhosphoSens* linearity data for PTP1B titration with AQT0266



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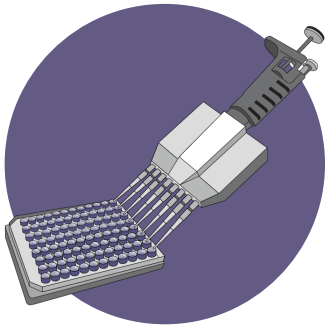
Products and Services Enabling Your Drug Discovery Needs

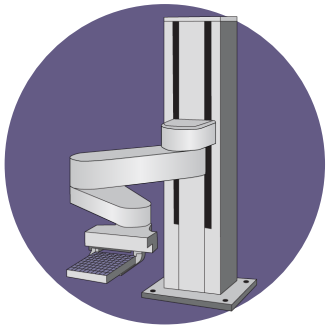
Catalog Kits and Bulk Reagents

Our direct, real-time, kinetic, enzyme activity assays **yield better, more reliable data** than other methods, enabling better decisions regarding target biology and generation of lead candidates to empower faster movement through the drug discovery process. Whether used in high, moderate, or low throughput modes, the resulting richer information from quantitative data allows you to rapidly rank candidates by inhibition mechanism, selectivity profiling, and multiple measures of potency, with covalent and reversible inhibitors and fragments.



Assay Development Services

AssayQuant has developed a highly reliable and robust process or developing new kinase and phosphatase activity and inhibitor assays, using our proprietary ~30,000-member library of Sox-based, fluorogenic substrates for each branch of the human kinome. Once the final PhosphoSens fluorogenic substrate's peptide sequence is identified, we scale up synthesis and HPLC-purify to create the final product, which can be purchased in kits and in bulk.



Compound Testing Services

Let us perform compound testing for you. Rely on our extensive experience with performing multiple types of determinations, our rapid turnaround times, and our detailed data analysis capabilities to accelerate your development of superior drugs. Identify lead candidates, profile your compounds, and empower your lead optimization process.

Contact us to see how we can support your drug discovery and development.

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