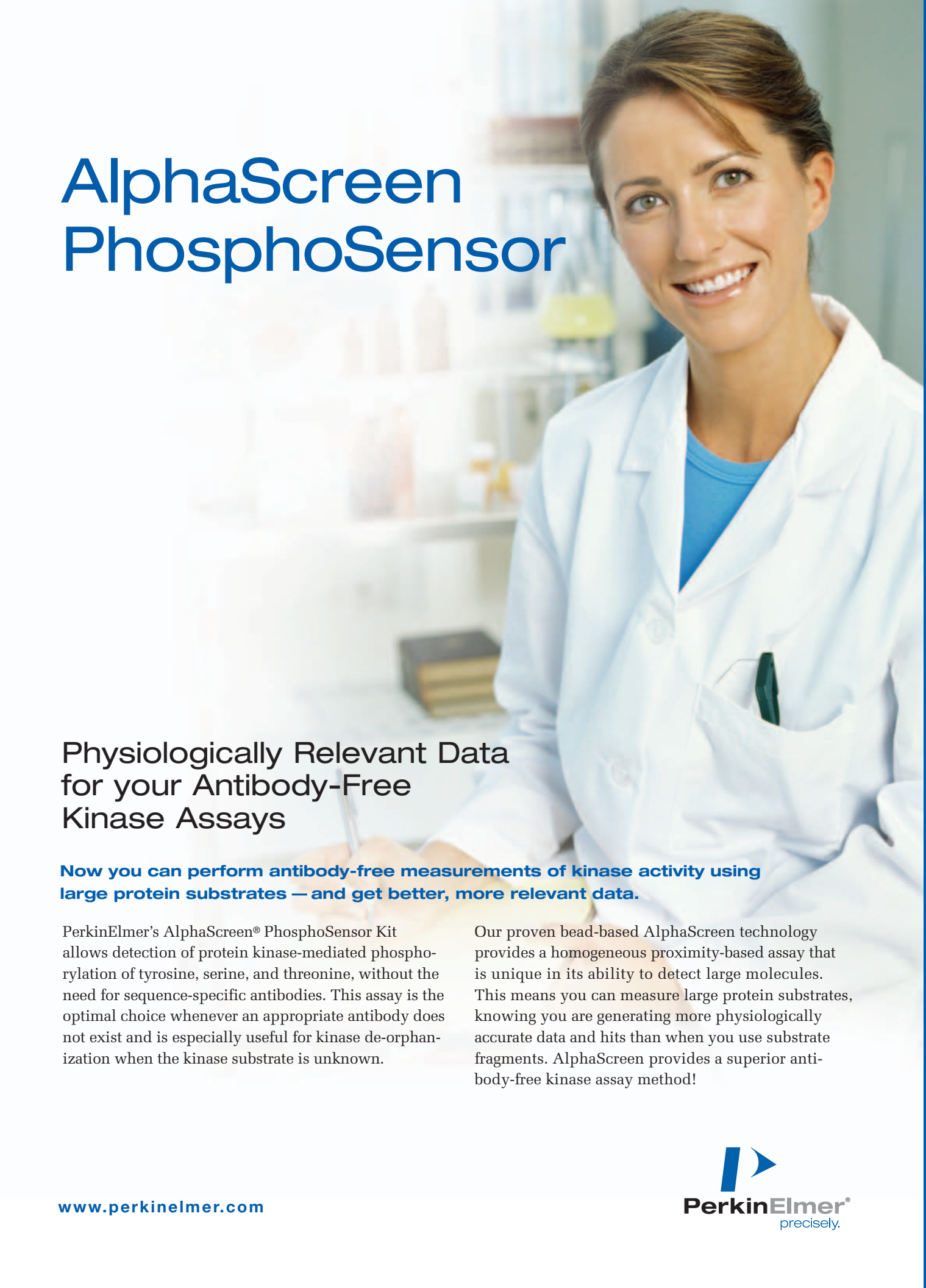


AlphaScreen PhosphoSensor

Physiologically Relevant Data for your Antibody-Free Kinase Assays

Now you can perform antibody-free measurements of kinase activity using large protein substrates — and get better, more relevant data.

PerkinElmer's AlphaScreen® PhosphoSensor Kit allows detection of protein kinase-mediated phosphorylation of tyrosine, serine, and threonine, without the need for sequence-specific antibodies. This assay is the optimal choice whenever an appropriate antibody does not exist and is especially useful for kinase de-orphanization when the kinase substrate is unknown.

Our proven bead-based AlphaScreen technology provides a homogeneous proximity-based assay that is unique in its ability to detect large molecules. This means you can measure large protein substrates, knowing you are generating more physiologically accurate data and hits than when you use substrate fragments. AlphaScreen provides a superior antibody-free kinase assay method!

www.perkinelmer.com

PerkinElmer®
precisely.

PerkinElmer offers the **most comprehensive set of too**
and screening. Our choice of technology offerings enables
substrate with the **utmost sensitivity and flexibility**, u

EnVision Multilabel Microplate Reader

Now available with sophisticated dispenser and temperature control options

Get the speed and capacity your Assay Development or HTS lab needs with the EnVision™ Multilabel Plate Reader from PerkinElmer. Our fast, application-oriented EnVision multilabel plate reader handles all non-isotopic detection technologies. It's even optimized for our robust and sensitive AlphaScreen technology.

EnVision's unique modular design lets you choose the optical modules for the detection technologies and applications you need to run. These optical modules provide the best possible performance for each and every label or application. To use AlphaScreen technology, choose the affordable AlphaScreen Standard Option module for assay development and medium throughput screening. Choose the AlphaScreen HTS Turbo Option module for high throughput screening.

Please visit **www.perkinelmer.com/platereaders** to view our free interactive EnVision tutorial.

Features & Benefits

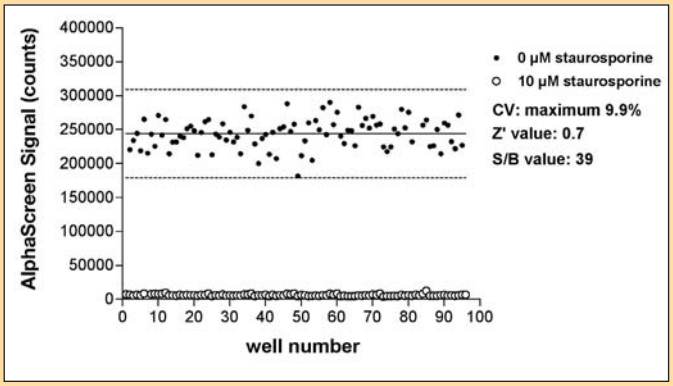
- **Antibody-free:** especially useful for ser/thr kinases for which no antibody exists.
- **Measurement of large protein substrates:** unlike with TR-FRET and fluorescence polarization technologies.
- **Excellent Z' values:** enabling successful transfer to HTS applications.
- **No fluorophore labeling of substrate**: simple biotinylation means you can cut your assay development time significantly.
- **Highly sensitive:** detects concentrations comparable to antibody-based systems.
- **Physiological assay conditions:** assays can be read at physiological pH to favor optimal protein substrate recognition, unlike fluorescent polarization technologies where detection can only be done in acidic conditions.
- **Ideal for kinase de-orphanization studies:** substrates need to be identified for over 500 kinases whose genes were uncovered during the human genome project.

"We developed AlphaScreen PhosphoSensor to fill a gap in kinase research and screening that only AlphaScreen technology could address — an ability to measure large protein substrates, antibody-free."

– Nathalie Rouleau
Senior Research Associate
PerkinElmer
Montreal, Canada

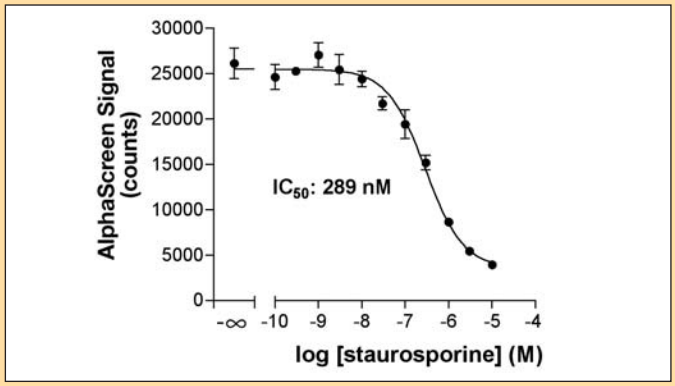
ls available from one source for **kinase research**
you to **screen for any kinase target** and **any size**
sing antibody-based or antibody-free systems.

Excellent Z' Values



Z' value determination for protein kinase A (PKA) using the AlphaScreen PhosphoSensor beads.

Measurement of Large Protein Substrates



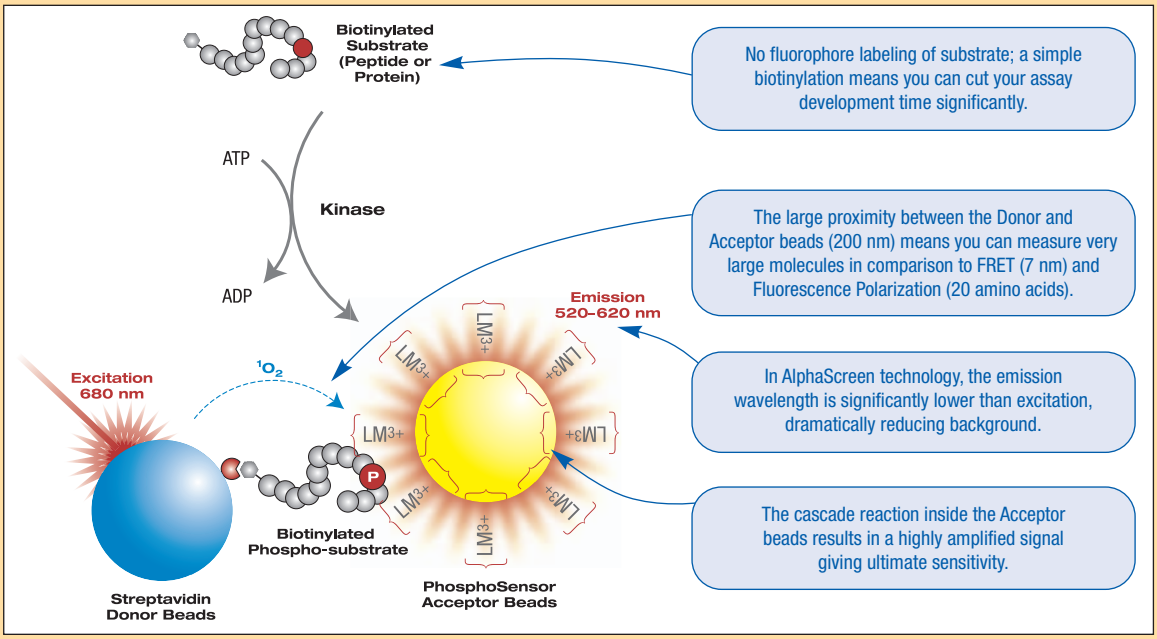
Antibody free detection of JNK3 kinase activity. The phosphorylation of a GST tagged version of cJun (amino acid 1 to 79) (GST-cJun), by recombinant JNK3, was detected using the AlphaScreen PhosphoSensor Acceptor beads.

How PhosphoSensor Kinase Assays Work

The assay is based on the use of streptavidin-coated Donor beads and PhosphoSensor Acceptor beads. The Acceptor beads are coated with a Lewis metal chelate that allows them to detect phosphate groups irrespective of the nature of the phosphorylated residues.

In the assay, the kinase-driven addition of a phosphate to a biotinylated substrate results in the simultaneous capture of the substrate by the PhosphoSensor Acceptor and the streptavidin Donor beads. Upon laser excitation of the Donor beads, the proximity of the Donor and PhosphoSensor Acceptor beads results in the production of an AlphaScreen signal between 520 and 620 nm. In the absence of phosphorylation, no signal will be generated.

When the biotinylated phosphorylated substrate binds to the Lewis metal chelate (LMC^{3+}) coating on the Acceptor beads and to the streptavidin coating on the Donor beads, the Donor and Acceptor beads are brought into close proximity. This generates an AlphaScreen signal. In the absence of binding, no signal is generated.



Ordering Information

AlphaScreen PhosphoSensor Kit

Product	Size	Part Number
AlphaScreen PhosphoSensor Kit	1,000 assay points	6760307D
AlphaScreen PhosphoSensor Kit	10,000 assay points	6760307M
AlphaScreen PhosphoSensor Kit	50,000 assay points	6760307R

Each PhosphoSensor kit includes PhosphoSensor Acceptor beads; Streptavidin Donor beads; Positive control bio-LCK-P and 10X control buffer.

Complementary Products

Optimization Tools

The AlphaScreen Omnibeads™ contain all the chemical components necessary for the generation of a strong AlphaScreen signal without requiring the presence of AlphaScreen Acceptor and Donor beads.

Product	Size	Part Number
AlphaScreen Omnibeads	1,000 assay points	6760626D
AlphaScreen Omnibeads	10,000 assay points	6760626M
AlphaScreen Omnibeads	50,000 assay points	6760626R

The AlphaScreen TruHits™ Kit is a tool for AlphaScreen users to identify false positives in AlphaScreen HTS assays early in the screening process and in a cost-effective way.

Product	Size	Part Number
AlphaScreen TruHits Kit	1,000 assay points	6760627D
AlphaScreen TruHits Kit	10,000 assay points	6760627M

AlphaScreen-Compatible Microplates

ProxiPlate™ Plus—Low volume, high performance 384-well plate: Perform miniaturized assays of 5–20 µL/well reducing reagent costs and conserving precious compounds, without sacrificing sensitivity.

Product	Size	Part Number
ProxiPlate-384 Plus, white, 384-shallow well	50/Box 200/Box	6008280 6008289

OptiPlate™—Standard high performance microplates: high quality microplates for luminescence applications.

Product	Size	Part Number
OptiPlate-96, white, 96-well	50/Box 200/Box	6005290 6005299
OptiPlate-384, white, 384-well	50/Box 200/Box	6007290 6007299
OptiPlate-1536, white, 1536-well	20/Box	6005228

PerkinElmer Expertise Delivers a Superior Antibody-Free Assay—plus a Complete Kinase Resource

For more detailed technical information about our superior antibody-free AlphaScreen PhosphoSensor and other PerkinElmer technologies for kinase research and screening, please visit **www.perkinelmer.com/drugdiscovery**.

PerkinElmer Life and Analytical Sciences
710 Bridgeport Avenue
Shelton, CT 06484-4794 USA
Phone: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

PerkinElmer® precisely.

For a complete listing of our global offices, visit www.perkinelmer.com/lasoffices

007530_02 Printed in USA