

AlphaScreen *SureFire* ERK Assay

A *SureFire*[™] answer to screening your toughest G_{αq}- and G_{αi}-coupled GPCR targets

The combination of PerkinElmer's highly sensitive, proven AlphaScreen[®] technology with TGR BioSciences' *SureFire*[™] cellular ERK assay takes screening of GPCR activation in whole cells to a new level of performance for traditionally challenging targets.

Now you can perform a homogeneous assay optimized for functional GPCR screening by measuring activated ERK 1/2 in cell lysates. The *SureFire*[™] assay is an excellent alternative for 'tough-to-screen' targets not optimally coupled through the calcium or cAMP pathways.

AlphaScreen is an extremely versatile non-isotopic homogeneous bead-based assay technology, field-tested in HTS and assay development. The *SureFire*[™] assay is the optimal choice when the highest sensitivity is required such as for analysis of GPCRs expressed at endogenous levels either in immortalized cell lines or in primary cells, or when common methods such as calcium or cAMP detection do not give screenable windows.

The assay is also an excellent choice as a secondary screening platform for hit confirmation.

AlphaScreen technology is unique in its ability to detect large full-length protein molecules, such as ERK 1/2, from relatively complex samples such as cell lysates.

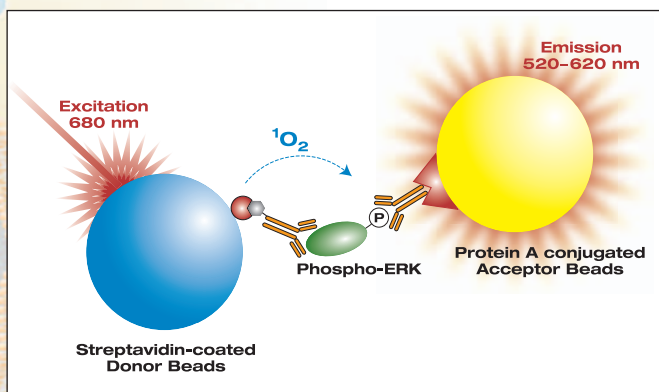
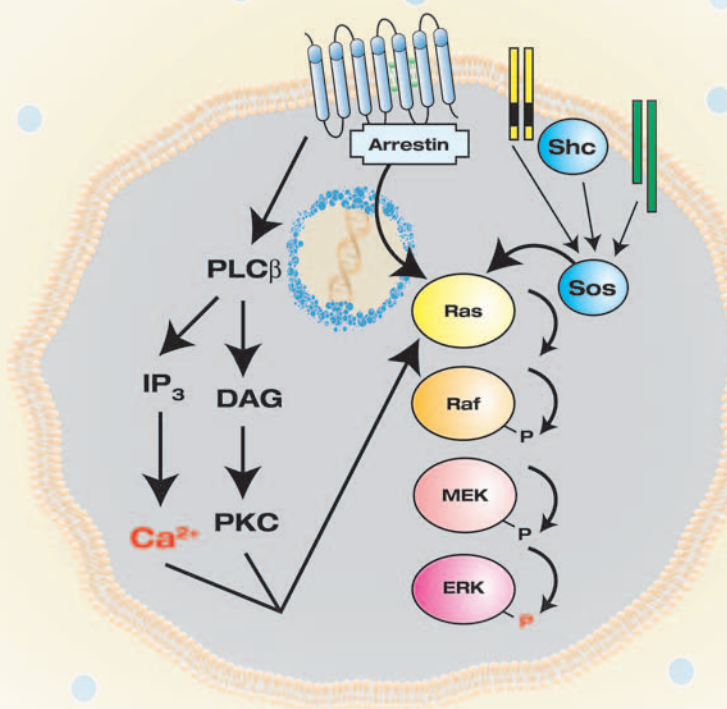
www.perkinelmer.com



Take your **GPCR** screening to a

Key Features & Benefits

- **Homogeneous cellular assay for ERK 1/2:** No-wash, non-isotopic, miniaturizable cell-based functional HTS assay for almost any GPCR including $G_{\alpha q}$, many $G_{\alpha i/o}$ - and some $G_{\alpha s}$ -coupled receptors.
- **Ideally suited for $G_{\alpha q}$ - and $G_{\alpha i}$ -coupled receptors:** Permits robust screening of high interest but traditionally challenging $G_{\alpha q}$ - and $G_{\alpha i}$ -coupled GPCR targets.
- **Excellent for 'tough-to-screen' GPCR targets:** Well suited for 'tough' targets not optimally coupled through the calcium or cAMP pathways.
- **Suitable for over-expressed and endogenous receptors:** Extremely sensitive assay that can be used with cells over-expressing cloned targets or with cells expressing physiologically relevant endogenous levels of receptor target.
- **Use in any cell line, including primary cells:** Flexible assay format and application use.
- **Easy to scale up and automate with excellent Z' values:** Utilizes HTS-proven AlphaScreen technology, which is easily scalable to 384- and 1536-well microplate formats, while yielding outstanding S/Bs and Z' values.



AlphaScreen SureFire™ ERK Assay Principle. Detection of activated ERK is enabled by immuno-sandwich capture of endogenous phosphorylated ERK in cell lysates. Antibody-coated AlphaScreen beads generate a highly amplified signal when in close proximity due to binding of phospho-ERK.

AlphaScreen Technology

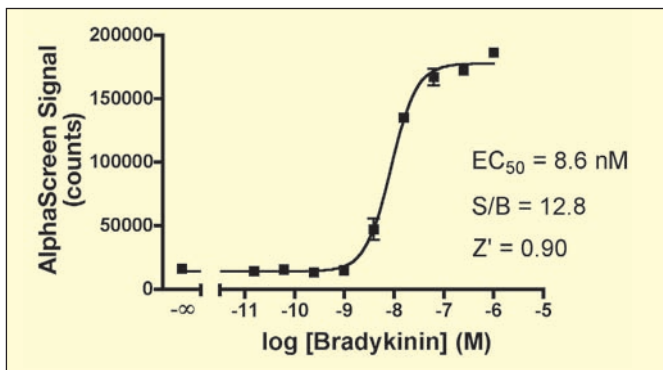
The AlphaScreen *SureFire™* ERK assay is just one of many useful screening tools built on PerkinElmer's versatile AlphaScreen technology. These assays are simple to set up and optimize so you are up and running quickly.

AlphaScreen is a homogeneous, bead-based platform widely used in assay development and high throughput screening. The long excitation wavelength (680 nm) combined with a shorter emission wavelength (520–620 nm) reduces interference from biological samples or assay components, ensuring a very low background. The result is very high sensitivity, at the low attomole level in many assays.

Unlike other proximity-based technologies, AlphaScreen beads can be up to 200 nm apart, allowing for the detection of very simple to very complex biological interactions, and substrates of many different sizes.

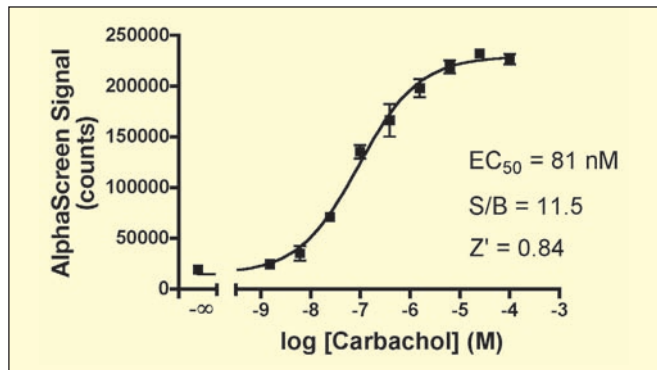
whole new level of performance

Stable Overexpressed $G_{\alpha q}$ -GPCR



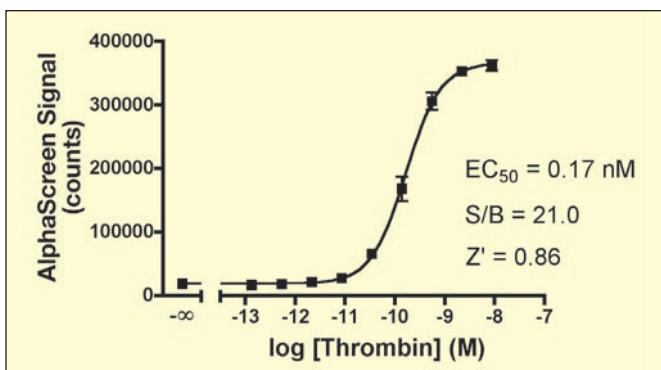
$G_{\alpha q}$ -coupled human Bradykinin R_2 receptor was overexpressed in HEK293 cells and stimulated with various concentrations of Bradykinin ligand. Phospho-ERK was measured in whole cell lysates using the AlphaScreen *SureFire*[™] ERK assay.

Stable Overexpressed $G_{\alpha i}$ -GPCR



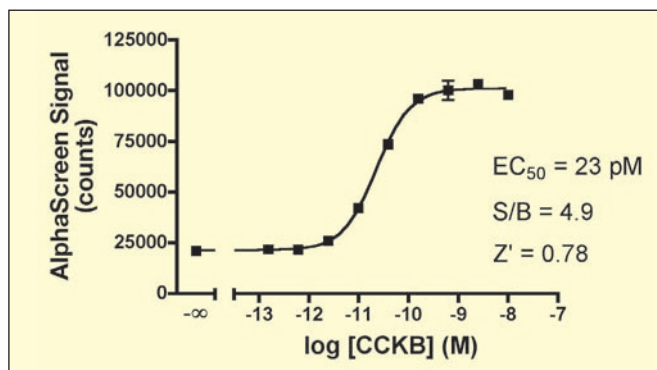
$G_{\alpha i}$ -coupled human Muscarinic M_4 receptor was overexpressed in CHO cells and stimulated with various concentrations of Carbachol ligand. Phospho-ERK was measured in whole cell lysates using the AlphaScreen *SureFire*[™] ERK assay.

Endogenously Expressed $G_{\alpha q}$ -GPCR



$G_{\alpha q}$ -coupled mouse Thrombin receptor, expressed at endogenous levels in Swiss 3T3 embryonic fibroblast cells, was stimulated with various concentrations of Thrombin ligand. Phospho-ERK was measured in whole cell lysates using the AlphaScreen *SureFire*[™] ERK assay.

Stable Overexpressed $G_{\alpha q}$ -GPCR



$G_{\alpha q}$ -coupled human CCK_2 receptor was overexpressed in HEK293 cells and stimulated with various concentrations of Cholecystokinin B (CCK_B) ligand. Phospho-ERK was measured in whole cell lysates using the AlphaScreen *SureFire*[™] ERK assay.

EnVision Multilabel Microplate Reader

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 reader handles all non-isotopic detection technologies. The EnVision is optimized for our robust and sensitive AlphaScreen technology. Choose the AlphaScreen HTS Turbo option module for high throughput screening.

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



Ordering Information

Product	Product No.	Size
SureFire™ ERK Kit	TGRES500	500 assay points
	TGRES10K	10,000 assay points
	TGRES50K	50,000 assay points
AlphaScreen Protein A Kit	6760617C	500 assay points
	6760617M	10,000 assay points
	6760617R	50,000 assay points

Note: Both SureFire™ ERK and AlphaScreen Protein A kits are required in equal quantities to run the ERK assay.

Complementary Products

AlphaScreen-Optimal Microplates

Product	Product No.	Size
Proxiplate-384 Plus, white, 384-shallow well	6008280	50/box
	6008289	200/box
OptiPlate-96, white, 96-well	6005290	50/box
	6005299	200/box
OptiPlate-384, white, 384-well	6007290	50/box
	6007299	200/box
OptiPlate-1536, white, 1536-well	6005228	20/box

AlphaScreen Optimization Tools

AlphaScreen Omnibeads are used for optimizing instrument set-up and in assay development.

AlphaScreen TruHits beads are ideal for counter screening and identifying false positives.

Product	Product No.	Size
AlphaScreen Omnibeads	6760626D	1,000 assay points
	6760626M	10,000 assay points
	6760626R	50,000 assay points
AlphaScreen TruHits Kit	6760627D	1,000 assay points
	6760627M	10,000 assay points

Get the SureFire™ answer to your toughest GPCR screening challenges

For more details about the AlphaScreen SureFire™ ERK Assay, visit www.perkinelmer.com/surefireerk.

For more information about SureFire™ technology, visit www.tgr-biosciences.com.au.

Visit www.perkinelmer.com/drugdiscovery, a comprehensive resource for researchers and scientists in drug discovery and development, and learn about PerkinElmer's broad offering of reagents and applications to support your research.

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