# AlphaScreen® *SureFire*®
# ERK 1/2 Total Assay Kits

# Manual

| Assay Points | Catalog # |
|---|---|
| 500 | TGRTESB500 |
| 10 000 | TGRTESB10K |
| 50 000 | TGRTESB50K |

For Research Use Only
Research Reagents for Research Purposes Only

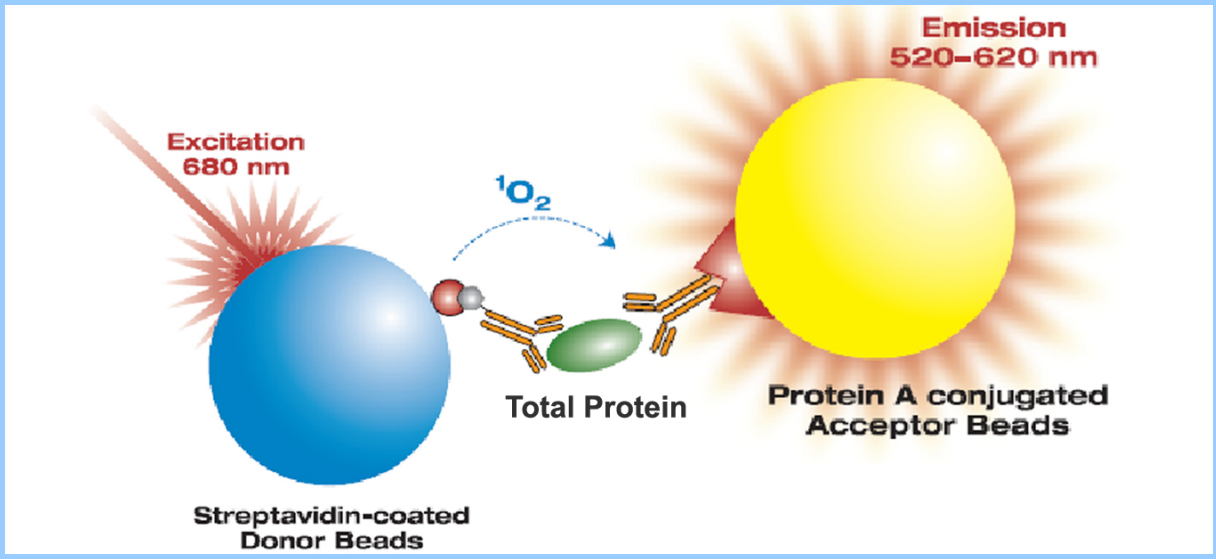
## General Information on the AlphaScreen® *SureFire*® ERK 1/2 assay

The AlphaScreen® *SureFire*® ERK 1/2 assay is used to measure endogenous ERK 1/2 in cellular lysates. The assay is an ideal system for normalizing ERK 1/2 levels for experiments measuring changes in ERK 1/2 phosphorylation, and can be applied to primary cells.

This assay eliminates the need for laborious techniques, such as Western blotting or conventional ELISA. It is a homogeneous assay, in that no sample washing steps are required, which allows for minimal handling, short assay times, and robotic operation if desired. The assay utilizes the bead-based Alpha Technology, and requires an Alpha Technology-compatible plate reader.

This new formulation (TGRTESB) has a monoclonal total antibody replacing a polyclonal total antibody in the previous kit (TGRTES), which delivers equivalent or better assay performance, and greater batch consistency.

## Alpha Technology AlphaScreen® *SureFire*® Assay Principle




AlphaScreen® *SureFire*® technology allows the detection of proteins in cellular lysates in a highly sensitive, quantitative and user friendly assay. In these assays, sandwich antibody complexes, which are only formed in the presence of analyte, are captured by AlphaScreen donor and acceptor beads, bringing them into close proximity. The excitation of the donor bead provokes the release of singlet oxygen molecules that triggers a cascade of energy transfer in the Acceptor beads, resulting in the emission of light at 520-620nm.

### Kit-Specificity information

This assay kit contains 2 antibodies which recognize two distinct epitopes on ERK1 and ERK2. The proteins detected by this kit correspond to GenBank Accessions NP 002737 (ERK1) and NP 620407 (ERK2). Alternate Names include p44 MAPK, MAPK3 (ERK1), p42 MAPK, MAPK1 (ERK2).

These antibodies recognize ERK 1/2 of human, mouse and rat origin. Other species should be tested on a case-by-case basis.

## Kit Contents

| | Kit Size | | |
|---|---|---|---|
| | **500 points** | **10,000 points** | **50,000 points** |
| Lysis buffer (5X) | 1 x 10 mL | 4 x 60 mL | 3 x 400 mL |
| Activation buffer | 1 x 2 mL | 1 x 60 mL | 1 x 300 mL |
| Reaction buffer | 1 x 2.6 mL | 1 x 45 mL | 1 x 225 mL |
| Dilution buffer | 1 x 1.5 mL | 1 x 25 mL | 2 x 60 mL |
| Assay Control Lysate | 1 tube to be re-dissolved in 250 µL $H_2O$ | | |

## Storage conditions upon receipt

The kit buffers e.g. 5X Lysis buffer, Activation buffer B and Reaction buffer should be stored at 4°C. DO NOT freeze the kit buffers – the Reaction buffer contains antibodies and freeze/thaw cycles can lead to a loss of activity.

## Materials Required But Not Provided

The AlphaScreen *SureFire* assay kits are optimized to work with AlphaScreen Protein A general IgG detection beads. These are available separately from PerkinElmer. The AlphaScreen Protein A general IgG detection kits contain a biotinylated rabbit IgG control, which can be used to test the instrument settings and bead performance.

| **Item** | **Suggested source** | **Catalog #** | **Size** |
|---|---|---|---|
| Protein A general IgG detection kit (contains the Acceptor and Donor Beads) | PerkinElmer Inc. | 6760617C<br>6760617M<br>6760617R | 500 pt<br>10,000 pt<br>50,000 pt |
| Proxiplate™-384 Plus, white, shallow well assay plate | PerkinElmer Inc. | 6008280<br>6008289 | 50/box<br>200/box |
| Optiplate™-384 Plus, white, assay plate | PerkinElmer Inc. | 6007290<br>6007299 | 50/box<br>200/box |
| TopSeal-A 384, clear adhesive sealing film | PerkinElmer Inc. | 6050185 | 100/box |
| Envision® or Enspire® Alpha-reader | PerkinElmer Inc. | - | - |

## Buffer preparation and subsequent storage conditions

| | |
|---|---|
| 5X Lysis buffer | Store 5X Lysis buffer at 4°C. For assay, dilute **5-fold** in water immediately prior to use. Discard unused buffer. |
| Activation buffer B | Precipitation will occur during storage 4°C. To re-dissolve, warm to 37°C and mix. Alternatively, Activation buffer can be stored at room temperature with no loss in activity. |
| Reaction buffer* | Keep on ice while in use. Do not freeze.<br>Once diluted discard unused reaction buffer. |
| AlphaScreen® Protein A IgG Kit | Store at 4°C in the dark. |
| **Acceptor Mix**<br>(Reaction buffer + Activation buffer + AlphaScreen® Acceptor beads) | Immediately prior to use, dilute Activation buffer B **5-fold** in Reaction buffer (e.g. take 98 µL Activation buffer B and dilute in 392 µL Reaction buffer).<br><br>Dilute Acceptor beads **50-fold** in Acceptor mix (e.g. add 10 µL Acceptor beads to 490 µL of premixed Reaction buffer + Activation buffer B).<br><br>The Acceptor mix should be used immediately for best results. Excess mix should be discarded. |
| **Donor Mix****<br>(Dilution buffer + AlphaScreen® Donor beads) | Immediately prior to use, dilute Donor beads **20-fold** in Dilution buffer (e.g. add 10 µL Donor beads to 190 µL Dilution buffer).<br><br>The Donor mix should be used immediately for best results. Excess mix should be discarded. |
| Assay Control lysate | After reconstitution in 250 µL water, lysates should be frozen at -20°C in single use aliquots and used within 1 month. |

* Do not vortex the Reaction buffer, as vigorous mixing can damage some antibodies.

** Prepare and use Donor Mix under low-light conditions.

## Control Lysate information

Control lysates are prepared from flasks of HEK293 cells (ATCC #CRL-1573) at a concentration of approximately 0.125 mg/mL. The controls are supplied lyophilized, and should be reconstituted in either dd $H_2O$ or MilliQ® $H_2O$. Once reconstituted, lysates should be stored frozen in single use aliquots.

Control Lysate: Prepared from flasks of untreated HEK293 cells. A serial dilution of assay control in 1X Lysis buffer should form a linear curve when assayed.

## Total ERK 1/2 AlphaScreen® *SureFire*® Assay Protocols

### A. 2-Plate Assay - assay protocol for adherent cells

*Cell Seeding*

1. Seed cells (200 µL of cells for 96 well plates, 50 µL for 384 well plates) in tissue culture plates. Incubate at 37°C overnight in serum-containing media.

*Cell Treatment*

2. Remove culture media, and stimulate the cells with 50 µL agonists prepared in serum-free media (25 µL for 384-well plates). (*If testing antagonists, prior to stimulation remove culture medium and replace with 50 µL serum-free media containing antagonists (25 µL for 384-well plates)).* Return cells to 37°C incubator for desired time. 1 hour is often sufficient for signal transduction inhibitors, and 5 minutes for receptor agonists.

**Note:** Peptidic agonists and antagonists can often stick to plastic surfaces. To minimize this effect, dilute in serum-free media containing a suitable carrier protein (e.g. 0.1% IgG free BSA - Jackson Immunoresearch Cat #001-000-161).

*Lysate Preparation*

5. To lyse cells, remove medium from wells, and add freshly prepared 1X Lysis Buffer (50-100 µL for a 96 well plate, 25 µL for a 384 well plate). Agitate on a plate shaker (~350 rpm) for 10 minutes at room temperature.

**Note:** If a more thorough lysis is required, the cells may be lysed with 1X Lysis buffer, containing Activation buffer B (mix 4 parts 1X Lysis buffer, 1 part Activation buffer B). Ensure that the Acceptor Mix is prepared WITHOUT Activation buffer B, if it is added with the Lysis buffer at this step.

6. Take 4 µL of the lysate and transfer to a 384-well Proxiplate™ for assay. (*Add 4 µL Control lysates to separate wells if required*).

*SureFire Assay*

7. Add 5 µL of Acceptor Mix to wells. Seal plate with Topseal-A adhesive film, and incubate for 2 hours at room temperature.

8. Add 2 µL of Donor Mix to wells under subdued light. Seal plate with Topseal-A adhesive film, and cover plate with foil. Incubate for 2 hours at room temperature.

**Note:** Longer incubation may give greater sensitivity. Plates can be incubated overnight if required.

9. Read plate on an Alpha Technology-compatible plate reader, using standard AlphaScreen settings.

**B. 1 Plate Assay - assay protocol for non-adherent cells, and for high-throughput applications.**

**Note: the larger volumes required using this assay will result in achieving less assay points per kit.**

*Cell Seeding*

1. Harvest cells by centrifugation, and re-suspend cells in HBSS at a suitable cell density. We recommend $10^7$ cells/mL as a starting point. Seed 4 µL of cells/well into a 384-well culture plate.

2. If using test agents/inhibitors, add 2 µL/well of 4X inhibitors prepared in HBSS.

**Note:** Peptidic agonists and antagonists can often stick to plastic surfaces. To minimize this effect, dilute in serum-free media containing a suitable carrier protein (e.g. 0.1% IgG free BSA - Jackson Immunoresearch Cat #001-000-161).

3. Return cells to incubator at 37°C for 1-2 hours.

*Cell Treatment*

4. Stimulate cells with agonists by addition of 2 µL/well of 4X agonist stock in HBSS containing 0.1% BSA. The final volume in the wells should be 8 µL. (*if no antagonists were used in step 2, stimulate the cells with 4 µL/well of 2X agonist, to give a final volume in the wells of 8 µL*.)

*Lysate Preparation*

5. To lyse the cells, add 2 µL/well 5X Lysis buffer. (*Add 10 µL control lysates to separate wells if required*).

**Note:** If a more thorough lysis is required, add 2 µL/well Activation buffer B to the cells, in addition to 2 µL 5X Lysis buffer. Ensure that the Acceptor Mix is prepared WITHOUT Activation buffer B, if it is added at this step.

*SureFire Assay*

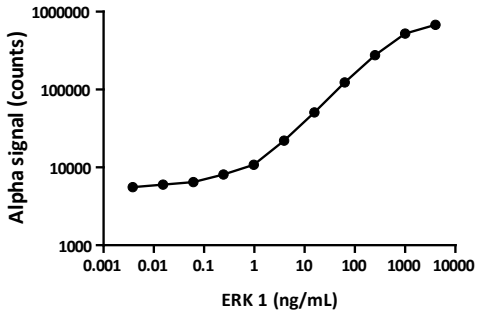
6. Add 8 µL of Acceptor Mix to wells. Seal plate with Topseal-A adhesive film, and incubate for 2 hours at room temperature.

7. Add 3 µL of Donor Mix to wells under subdued light. Seal plate with Topseal-A adhesive film, and cover plate with foil. Incubate for 2 hours at room temperature.

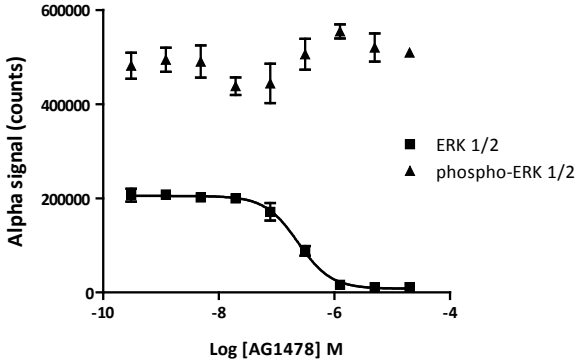
**Note:** Longer incubation may give greater sensitivity. Plates can be incubated overnight if required.

8. Read plate on an Alpha Technology-compatible plate reader, using standard AlphaScreen settings.

## Representative Data



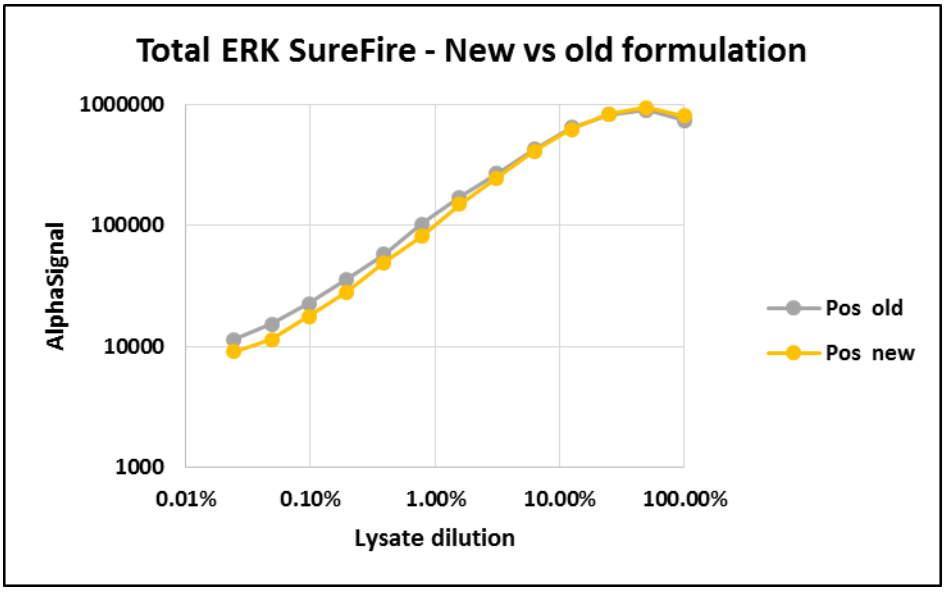

A dilution series of recombinant active ERK (Biaffin GMBH Cat# PK-ERK1-A010) was prepared in 1X Lysis buffer containing 0.1% IgG-free BSA, and analyzed for total ERK 1/2 assay using the standard 2-plate protocol, the limit of detection is around 1 ng/mL.




A431 cells were seeded into 96-well microplates, and cultured until confluent. The cells were treated with various concentrations of the EGFR inhibitor AG1478 for 2 hours. The medium was removed from the wells, and the cells were lysed with a combination of 1X Lysis buffer and Activation buffer B. The lysates were analyzed for both total ERK 1/2 and phospho-ERK 1/2, using the standard AlphaScreen *SureFire* 2-plate protocol.

Comparison of new SureFire Total ERK (TGRTESB) vs old p-ERK (TGRTES) Assay kit performance

Control lysates were diluted serially in 1X Lysis buffer and assayed with the respective kits in a 2-plate assay format. S:B of the new TGRTESB kit was 135 vs TGRTES of 104.



## Frequently Asked Questions & Troubleshooting

For comprehensive information on assay optimization and troubleshooting, please refer to the following resources:

- Guide to AlphaScreen® *SureFire*® assay optimization
- AlphaScreen® *SureFire*® user guide

To download these resources, and other related technical information, visit http://www.perkinelmer.com/category/alpha-surefire-kits
For general information on AlphaScreen® *SureFire*® assays, visit http://www.tgrbio.com

## Customer Care

To contact the customer care team, please visit **www.perkinelmer.com/ServiceCall**

**For more information regarding related AlphaScreen® *SureFire*® products and protocols refer to:**

**PerkinElmer web site: www.perkinelmer.com**

**TGR BioSciences website: www.tgrbio.com**

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