

An AlphaScreen® SureFire® Phospho-ERK1/2 assay performed on BMG LABTECH's POLARstar Omega

J.D. Brown, R. Mould and C.J. Langmead
Department of Pharmacology, Heptares Therapeutics, Welwyn Garden City, UK

Application Note 210, Rev. 01/2011

- ERK phosphorylation cascade can be utilized to measure Gi and Gq GPCR
- The AlphaScreen® SureFire® Phospho-ERK1/2 assay was performed on the POLARstar Omega in 384-well format
- Dose response curves for Gi and Gq agonists are shown

Introduction

G-protein coupled receptors (GPCRs) are a large family of receptors which have a broad involvement in cellular responses affecting many important body functions both in health and disease. On activation GPCRs signal through different G-proteins (e.g. Gs, Gi or Gq) to elicit a cellular response which can be mediated through the activation of a number of different signaling cascades which include intracellular Ca²⁺ mobilization and the formation or inhibition of adenylate cyclase. Although these technologies have been successful in many cases they have had their limitation. Responses for many GPCRs such as Gi coupled GPCRs are not easily measured in current assay formats and other methods have been investigated as alternative readout. One such pathway is the ERK phosphorylation cascade, which results in the phosphorylation of both cytoplasmic and nuclear proteins regulating gene transcription. Hence this type of assay has the ability to measure cellular changes activated by Gs, Gi and Gq coupled receptors giving potential to use a single assay format for screening.

Here the POLARstar Omega is used to detect ERK phosphorylation using a AlphaScreen® SureFire® proximity-based assay. ERK phosphorylation is demonstrated at receptors which signal via Gi and Gq G-proteins.

Assay Principle

The AlphaScreen® assay uses the diffusion of singlet state oxygen from Donor to Acceptor beads. Upon excitation at 680 nm of Donor beads ambient oxygen is converted into singlet oxygen released at a rate of up to 60,000 molecules per second. Singlet oxygen molecules have a short lifetime (4 μs in aqueous solutions) and diffuse of no more than 200 nm. When a biomolecular interaction brings the Donor and Acceptor beads in proximity, the singlet oxygen reaches the Acceptor bead and a cascade of chemical reactions is initiated producing a greatly amplified luminescence signal in the range of 520 – 620 nm. The AlphaScreen® SureFire® ERK1/2 phosphorylation assay is based on an immuno-sandwich assay principle (Figure 1).

A phosphorylated cellular protein (analyte) is sandwiched between a streptavidin (SA)-coated Donor bead associated with an analyte antibody and a Protein A-conjugated Acceptor bead associated with an anti-phospho specific antibody. Phosphorylation of the analyte results in an increase in the luminescence signal.

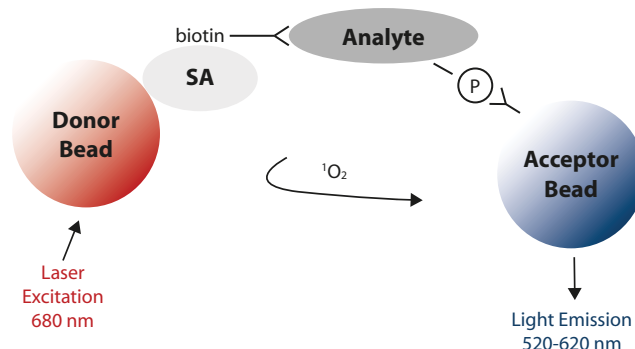


Fig. 1: Assay Principle of the AlphaScreen® SureFire® Phospho-ERK1/2 assay

Materials and Methods

- POLARstar Omega, BMG LABTECH, Offenburg, Germany
- AlphaScreen® SureFire® ERK1/2 phosphorylation assay kit, PerkinElmer, USA, #TGRES10K
- AlphaScreen® Protein A general IgG detection kit, PerkinElmer, USA, #6760617M
- Proxiplate™ -384 plus, white, 384-shallow well plate, PerkinElmer, USA, #6008280
- 96-well half area tissue treated cell culture plate, Corning, USA, #3688

CHO cells stably expressing either a Gq or Gi coupled GPCR were plated at a cell density of 25K per well in 96 well half area tissue culture treated plates and cells were allowed to attach for 4h at 37°C. After 4h media was removed and replaced with 45 μL of DMEM/F12 w/o FBS to allow quiescence of the cells. Cells were incubated for 18h and then the assay performed. 5 μL of test compound was added and incubation time varied for time course experiments while kept constant, 5min, for concentration response curve experiments. Following the incubation period, culture medium was removed and cells lysed with lysis buffer. Lysates were then transferred to a white 384-shallow well Proxiplate™ and AlphaScreen® beads were added in a single pipetting step in accordance with the kit protocol (1). The plate was incubated for 2h and read on the POLARstar Omega. Data were expressed as % maximal response obtained with 0.3% FBS.

Instrument Settings

Measurement method: AlphaScreen
Reading Mode: Endpoint
Filters: Ex AS/Em AS
Optic: 2 mm top optic

General Settings

Positioning delay: 0.50 s
Excitation time: 0.30 s
Integration start: 0.34 s
Integration time: 0.60 s

Results and Discussion

Initial experiments were designed to look at the optimal time of stimulation for both receptors.

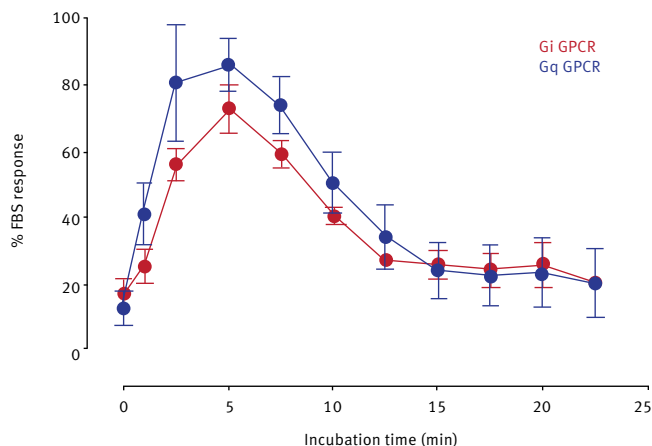


Fig. 2: FBS response dependent on incubation time

Agonist stimulation gave a transient increase in pERK1/2, peaking at 5 min for both Gi and Gq receptor activation.

Using the 5 min stimulation time point standard agonists were profiled in the concentration response curve format.

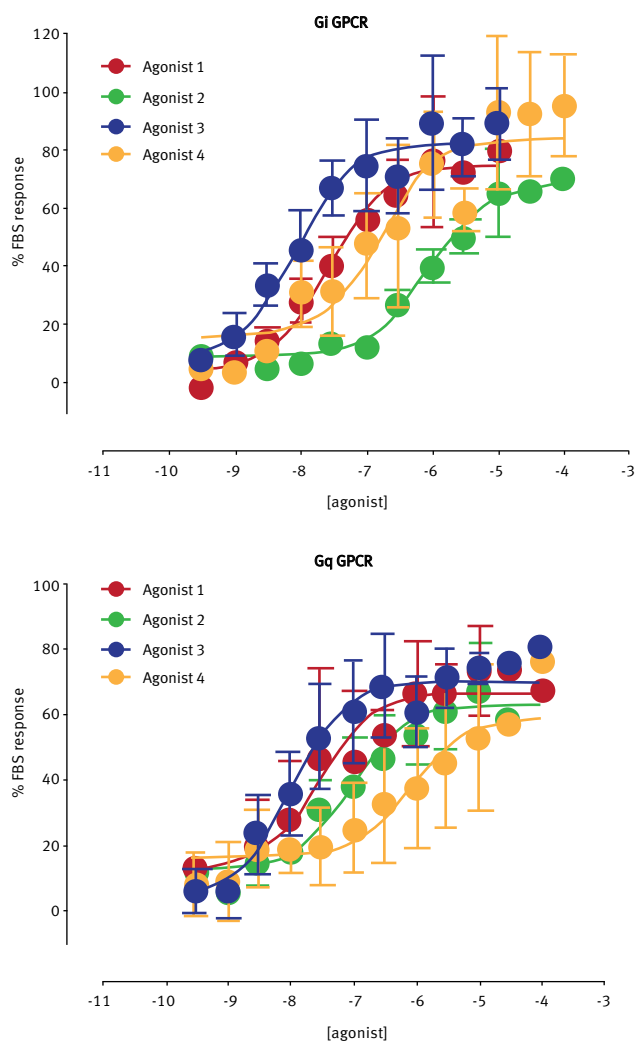


Fig. 3: AlphaScreen® measurement of activated Gi and Gq coupled GPCRs.

The AlphaScreen® SureFire® ERK1/2 phosphorylation assay system is able to detect G-Protein coupled receptor signaling and can be robustly measured using the POLARstar Omega microplate reader.

Conclusion

The AlphaScreen® SureFire® ERK1/2 phosphorylation assay was successfully performed on a POLARstar Omega in 384-well format. Although there is no laser available, special filters that are optimized for AlphaScreen® provide the possibility to obtain sensitive and consistent data.

The POLARstar Omega is a versatile, automated microplate reader that offers a range of detection modes: UV/VIS absorbance spectra, fluorescence intensity, fluorescence polarization, time-resolved fluorescence, time-resolved FRET, luminescence (flash and glow) and AlphaScreen®. Top and bottom plate reading, well scanning, precise temperature control and multi-mode shaking capabilities enhance the flexibility of the reader.

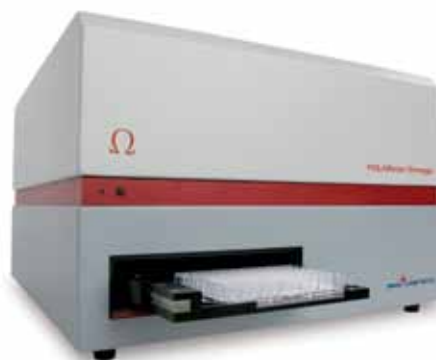


Fig. 4: BMG LABTECH's multidetection microplate reader POLARstar Omega.

The Omega instruments are also certified for the Dual Luciferase Reporter (DLR™) gene assay, for the HTRF® assay platform, and has the LanthaScreen™ certified plus status.

References

1. AlphaScreen® SureFire® ERK1/2 phosphorylation Assay Kit Protocol #TGRES10K, PerkinElmer, USA.

AlphaLISA and AlphaScreen are registered trademarks of PerkinElmer, Inc. HTRF is a registered trademark of Cisbio International. DLReady is a trademark of Promega Corp. LanthaScreen is a registered trademark of Invitrogen Corp.

Germany:	BMG LABTECH GmbH	Tel: +49 781 96968-0
Australia:	BMG LABTECH Pty. Ltd.	Tel: +61 3 59734744
France:	BMG LABTECH SARL	Tel: +33 1 48 86 20 20
Japan:	BMG LABTECH JAPAN Ltd.	Tel: +81 48 647 7217
UK:	BMG LABTECH Ltd.	Tel: +44 1296 336650
USA:	BMG LABTECH Inc.	Tel: +1 877 264 5227
Internet:	www.bmglabtech.com	applications@bmglabtech.com