Technical Note

Setting up AlphaScreen cAMP Assays With the Thermo Scientific Varioskan LUX Multimode Reader

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Goal

This application note describes how to perform AlphaScreen assays with the Thermo Scientific™ Varioskan™ LUX multimode microplate reader. The procedure and performance of the system are demonstrated below using the AlphaScreen cAMP assay, following the general procedure for the assay kit optimization.

Introduction

G-protein-coupled receptors (GPCRs) are involved in a large amount of physiological processes, where cyclic AMP (cAMP) is a key second messenger in intracellular signal transduction.

The AlphaScreen cAMP assay is used to measure levels of cAMP produced upon modulation of adenylate cyclase activity by GPCRs. The assay is based on the competition between endogenous cAMP and exogenously added biotinylated cAMP (Figure 1).

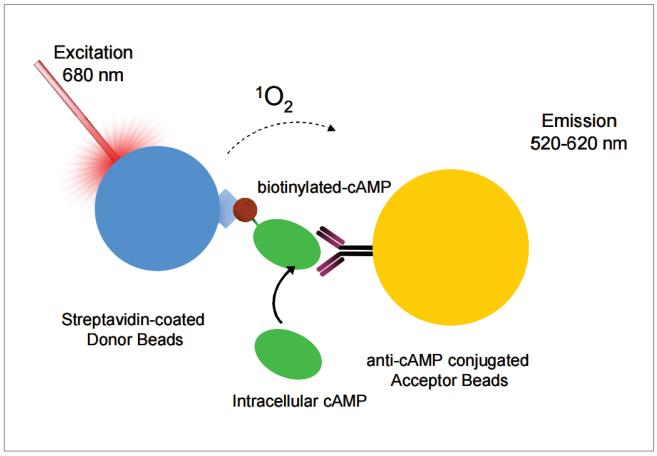

Figure 1: Principle of the AlphaScreen cAMP assay (Reference 1).

Cells can be stimulated to either increase or decrease intracellular cAMP levels. For Gαi-coupled receptors, an elevation in intracellular cAMP can be stimulated using forskolin. This causes a decrease in the AlphaScreen signal because of an inhibition of association between the beads. The measurement of cell-based protein-protein interactions using AlphaScreen technology requires cell lysis.

This paper describes how to set-up the AlphaScreen cAMP assay with the Varioskan LUX multimode reader. The process contains a biochemical assay with cAMP standards and a cell-based dose response assay. The calculations needed for data analysis of both of the assays are also demonstrated.

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Materials and methods

- Varioskan LUX multimode microplate reader, (Thermo Fisher Scientific)
- Forskolin (Sigma-Aldrich®, F688610MG)
- CHO-1 cells
- IBMX (3-Isobutyl-1-methylxanthine) (Sigma-Aldrich I5879-100MG)
- White Optiplate 384 (Perkin Elmer® 6007290)

The assay was performed according to Perkin Elmer functional cAMP assay instructions (Reference 1). Two parts of the process—the quality control and forskolin dose response—are reported in this note.

The measurement protocol was set up with the Thermo Scientific™ SkanIt™ software, a PC software for controlling the Varioskan LUX multimode reader. The optimized default parameters for AlphaScreen assays were used for all measurements (Figure 2).

The excitation wavelength of Varioskan LUX is fixed to 680 nm for Alpha technology, therefore only the emission filter needs to be chosen according to the chemistry used, i.e., AlphaScreen vs AlphaLISA.

The filter used for AlphaScreen is 571 nm with a very wide (77 nm) bandwidth.

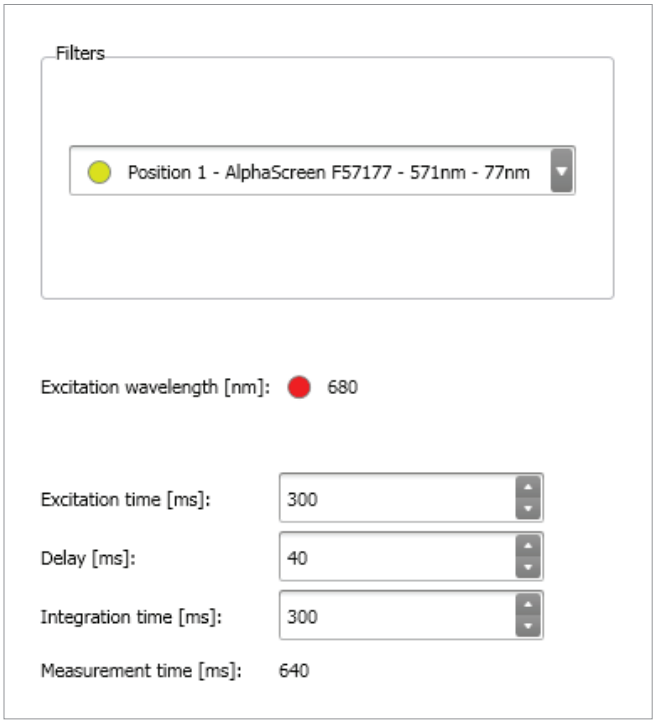

Figure 2: AlphaScreen protocol set-up with SkanIt software.

Users can adjust all of the parameters, including increasing or decreasing the measurement speed for various throughput requirements. A shorter measurement time naturally has an effect on the assay performance, but in high throughtput screens this may be a valuable feature. It is also possible to lengthen the times to gain even better precision.

The two parts of the process are reported separately below.

1. cAMP STANDARD CURVE PROCEDURE (QUALITY CONTROL MODE)

The test was performed according to Section V of Reference 1.

An 11-point dilution series of cAMP ($1x10^{-11}$–$5x10^{-6}$ M) was made to the kit control buffer. A no cAMP positive control was also prepared.

5 µL anti-cAMP Acceptor beads solution, 5 µL cAMP dilutions and 15 µL biotinylated cAMP/streptavidin Donor beads detection mix were pipetted to the assay plate. All samples were assayed in triplicates.

The plate was incubated in dark for 1 hour and measured with Varioskan LUX using the parameters reported above.

2. FORSKOLIN DOSE-RESPONSE

The test was performed according to the Section VII of Reference 1.

Forskolin dilution series with concentration range from $2x10^{-4}$–$2x10^{-9}$ M was made to stimulation buffer containing IBMX. No cell – no cAMP and no cell – 1µM cAMP controls were included.

10,000 CHO-M1 cells/well were pipetted to the plate, after which the kit reagents and the forskolin dilutions were pipetted as instructed in the kit documentation, and the AlphaScreen signal was measured with Varioskan LUX.

Results

1. cAMP STANDARD CURVE PROCEDURE

In the figure below, the AlphaScreen signal is plotted as the function of the cAMP concentration using the four parameter logistic fit of SkanIt software.

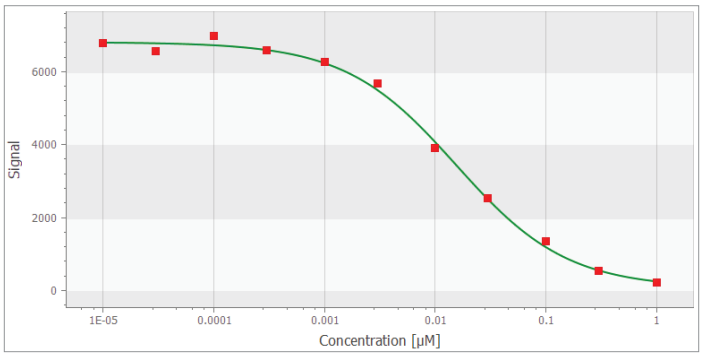

Figure 3: cAMP standard curve.

The signal to background ratio calculated from no-cAMP and 1 µM cAMP standard was ≈36 and the calculated ED50 value was 16 nM. Those values correlate well with the values reported on the reference 1: 12.5 and 7.5-10 nM, respectively. The signal to background exceeds the reference value dramatically.

2. FORSKOLIN DOSE-RESPONSE

The forskolin dose response results were calculated by the SkanIt software, which was also used to generate the dose response curve and to calculate the ED50 value (Figure 3).

The ED50 value is a concentration in which 50% of the monitored characteristic of the sample has been lost compared to the reference sample (B0). Calculation is always made from a normalized data set. In this case the data was normalized to the sample with the highest forskolin concentration.

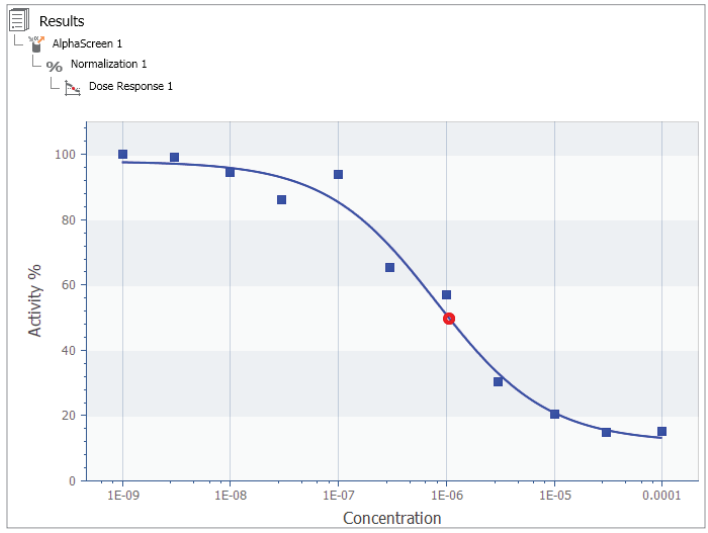

Figure 4: Forskolin dose response graph created by SkanIt software. AlphaScreen signal is plotted as the function of forskolin concentration. ED50 value is marked in red on the graph.

Z' is a dimensionless parameter, which describes the quality of a screening assay. The value ranges from 0-1 and assays with values above 0.5 can be considered very good. In this case the Z' calculated from no cAMP ja 1 µM cAMP controls was 0.82, which is excellent.

Conclusions

Varioskan LUX provides both easy-to-use and high performance tools for AlphaScreen assays.

The system contains optimized parameters for all of the measurement technologies, but also provides the opportunity to adjust the measurement parameters for assay set-up and optimization. Coupled with the in-built calculations of SkanIt software, this makes the system a very flexible tool from research to high-throughput screening.

References

Perkin Elmer Life and Analytical Sciences. (February 4, 2015). *Performing AlphaScreen cAMP Functional Assays.* www.perkinelmer.com.

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