HTRF® IP-One Terbium-based assay performed on BMG LABTECH´s PHERAstar *Plus*

Franka Ganske, BMG LABTECH, Germany
Laurence Jacquemart, Alexandre Jean, Marie-Laure Lebreton and Jean-Luc Tardieu, Cisbio Bioassays, France

Application Note 177 Rev. 09/2008

- HTRF® IP-One Tb is the unique HTS assay to investigating all the classes of compound of Gq activation pathway
- Better sensitivity and wider assay window
- Successfully performed on the PHERAstar and PHERAstar *Plus*

Introduction

Inositol-1,4,5-triphosphate (IP3) is an important second messenger in the cell. It is synthesized during cascade reactions that happen after stimulation of a G-protein coupled receptor (GPCR).[1] (Figure 1)

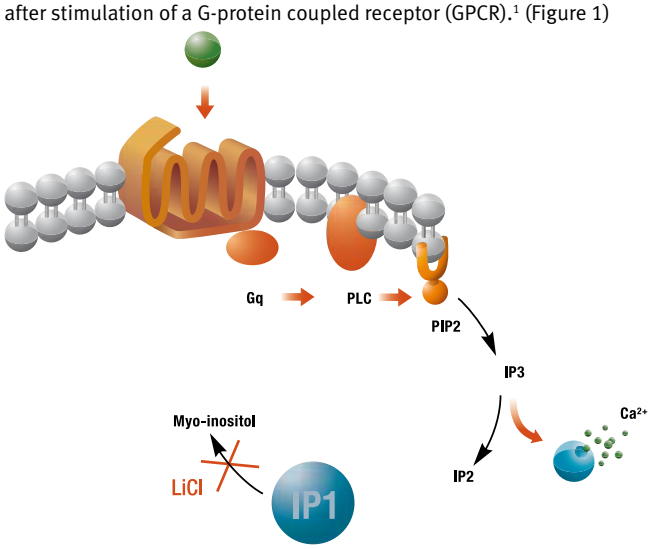

Fig. 1: Biosynthesis of IP1

After activation of a G-protein coupled receptor phospholipase C (PLC) is released. This lipase cleaves the membrane lipid phosphatidylinositol-bisphosphate (PIP2) into diacylglycerol and IP3. The main function of IP3 is to open calcium channels of the endoplasmic reticulum (ER) leading to the release of calcium ions into the cell.[2] The possibility to determine the IP3 concentration would allow for studying potential activators or inhibitors of the G-protein coupled receptors. However, the IP3 molecule has extremely short half life, making it difficult to use as a measure of Gq receptor activation. It turned out that in presence of LiCl the downstream metabolite IP1 is stable and accumulates.[3]

Cisbio Bioassays developed highly accurate HTRF® assays for measuring IP1 in 96-, 384- and 1536-well formats providing HTS compatible assays. HTRF® (homogeneous time-resolved fluorescence) is based on a fluorescence resonance energy transfer between a Europium cryptate (donor) and a second fluorescent label (acceptor). Recently Cisbio Bioassays developed a new Terbium cryptate (Lumi4™) that acts as a FRET donor. In this application note we show the comparison of BMG LABTECH's PHERAstar and PHERAstar *Plus* microplate readers to measure the new Terbium-based IP-One Tb assay.

Assay Principle

The IP-One assays are competitive immunoassays that use Terbium cryptate-labeled anti-IP1 MAb and d2-labeled IP1. (Figure 2).

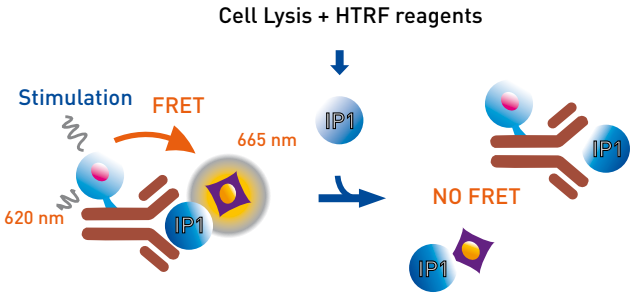

Fig. 2: IP-One assay principle

The assay is based on a monoclonal antibody specific for IP1, labeled with the new Lumi4™-Tb cryptate. This antibody competes with native IP1 produced by cells and IP1 coupled to the dye d2. The specific emission signal is inversely proportional to the concentration of IP1 in a standard or in the cell lysate.

Materials and Methods

- IP-One Tb Assay Kit, Cisbio Bioassays, France
- Black and white 384 small volume microplates, Greiner, Germany
- PHERAstar and PHERAstar *Plus*, BMG LABTECH, Germany (Figure 3)

Standard curve measurements were carried out using the IP1 calibrator that was sent together with the assay kit. After distribution of reagents (20 µL final volume) according to the kit manual, the plate was incubated for 1 h at room temperature and afterwards time-resolved fluorescence at 620 nm and 665 nm was measured on the PHERAstar and the PHERAstar *Plus* using the HTRF® optic module. The signal is stable over a 24 h period time at room temperature.

Fig. 3: BMG LABTECH's multidetection plate reader PHERAstar *Plus*

Data reduction

As for all HTRF® assays, data reduction using the fluorescence ratio (665 nm/620 nm) eliminates possible photophysical interferences and means the assay is unaffected by the usual buffer conditions and colored compounds. Results are calculated as follows:

Delta F % = [(Standard or sample Ratio – $Ratio_{neg}$) / $Ratio_{neg}$] x 100

Results and Discussion

Figure 4 shows the IP1 standard curves for the PHERAstar and the PHERAstar *Plus* in black 384-well small volume plates.

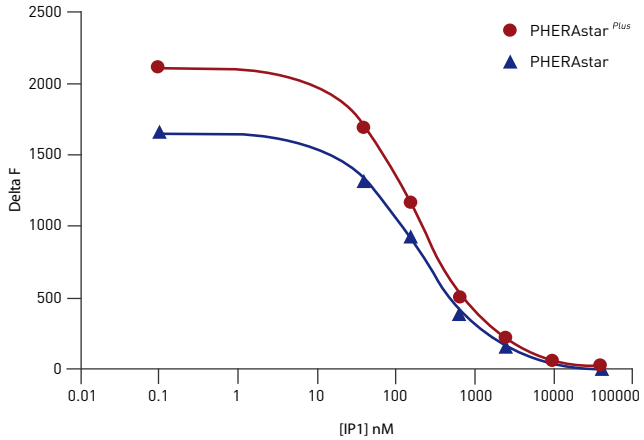

Fig. 4: IP1 standard curve obtained in black 384 well plates (volume = 20 µL) for the PHERAstar and the PHERAstar *Plus*

The data shows the great improvement for the PHERAstar *Plus* in HTRF® mode. The assay window and the sensitivity (EC_{50}) are greatly improved using Lumi4™ Terbium cryptate instead of Europium cryptate: assay window of IP-One Eu is 2.48, whereas the assay window for IP-One Tb is 4.92. (Figure 5)

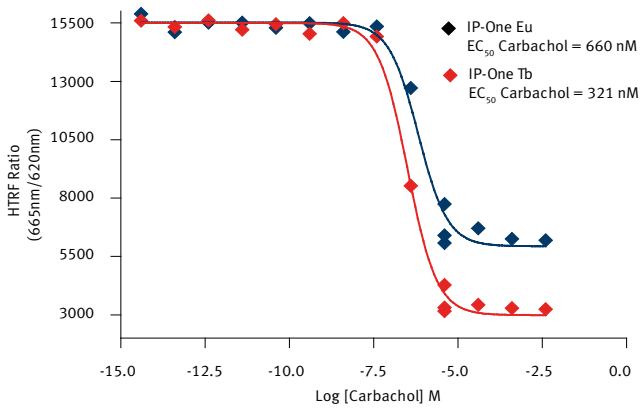

Fig. 5: IP-One Eu assay versus IP-One Tb assay performed on the PHERAstar *Plus* using CHO-M1 cells (30,000 cells/well-384 wells) that were stimulated with carbachol.

In order to compare the stability of the IP-One Tb and IP-One Eu Assays, the Z'factor was calculated.

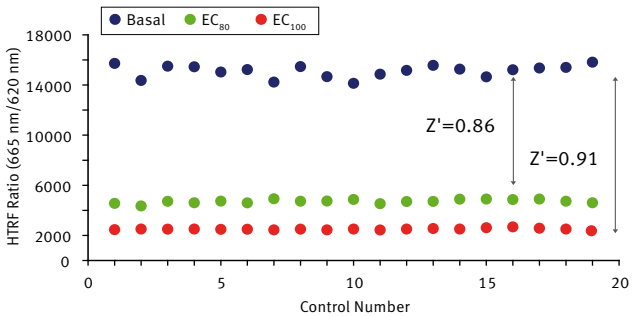

Fig. 6: IP-One Tb assay performed on the PHERAstar *Plus* using CHO-M1 cells (30,000 cells/well-384 wells) that were stimulated with carbachol at EC_{80} and EC_{100}. The basal activity was used for Z'calculation.

The Z' value of the IP-One Eu found at 0.77 increased to 0.91 for the IP-One Tb. This shows a better assay robustness.

Conclusion

With the new IP-One Tb assay Cisbio Bioassays developed an improved assay that shows a better sensitivity and a wider assay window.

This Terbium-based IP-One assay was successfully performed on the PHERAstar and the PHERAstar *Plus*. Compared to the forerunner instrument the PHERAstar *Plus* offers advanced HTRF® performance and the simultaneous dual emission (SDE) detection provides highest sensitivity and fast read times. The PHERAstar *Plus* is a microplate reader developed for HTS assays and offers full modularity in all detection modes.

References

1] Liu, K., Titus, S., Southall, N., Zhu, P., Inglese, J., Austin, C.P. and Zheng, W. (2008) Comparison on Functional Assays for Gq-Coupled GPCRs by Measuring Inositol Monophospate-1 and Intracellular Calcium in 1536-Well Plate Format. *Curr. Chem. Genomics*, **1,** 70-78.

2] Cassutt, K.J., Orsini, M.J., Abousleiman, M., Colone, D. and Tang, W. (2007) Identifying nonselective hits from a homogeneous calcium assay screen. *J. Biomol. Screen.*, **12** (2), 285-287.

3] Trinquet, E. et al. (2006) D-myoinositol 1-phosphate as a surrogate of D-myo-inositol 1,4,5-tris phosphate to monitor G protein-coupled receptor activation. *Anal. Biochem.*, **358** (1): 126-135.

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: +1 919 806 1735
Internet:	**www.bmglabtech.com**	**info@bmglabtech.com**