Functional Screening of fresh and frozen recombinant CHO-M1 cells

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Introduction

G protein coupled receptors (GPCR) are a most prominent group of therapeutic targets. In most cases, the activation of GPCRs results either in the alteration of cellular cAMP level or in the release of calcium ions from intracellular stores. (Figure 1)

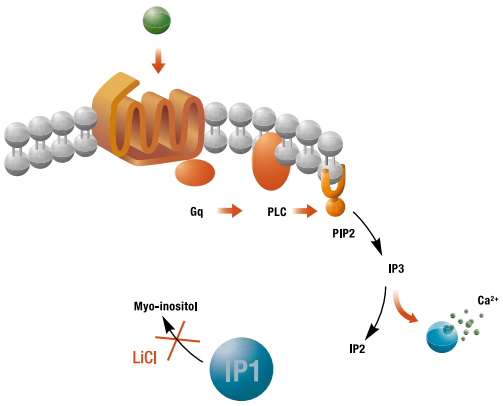

Fig. 1: Biosynthesis of IP1

Various assays are available to determine receptor activation by direct or indirect quantification of these second messengers.

Cisbio Bioassays developed highly accurate HTRF® assays for measuring second messengers like IP1 or cAMP in HTS formats. HTRF® (homogeneous time-resolved fluorescence) is based on a fluorescence resonance energy transfer between a Europium cryptate (donor) and a second fluorescent label (acceptor).[1]

This application note demonstrates the use of the IP-One HTRF® assay for a functional selection of clones from stably transfected recombinant CHO-M1 cells. The measurements were performed on BMG LABTECH's PHERAstar.

Assay Principle

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

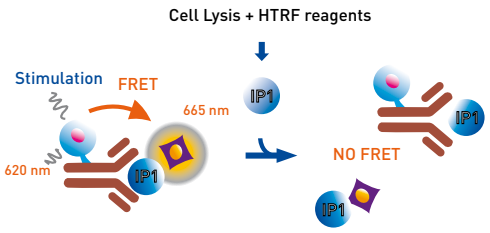

Fig. 2: IP-One assay principle

The assay is based on a monoclonal antibody specific for IP1, labeled with europium or terbium 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 & Methods

- IP-One Assay Kit, Cisbio Bioassays, France (#62P1APEB)
- Fresh and Frozen Cell lines, CCS Culture Service, Germany
- PHERAstar microplate reader, BMG LABTECH, Germany (Figure 3)

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

Isolation and screening of single cell clones

CHO-K1 cells were transfected with an expression vector encoding the Gαq-coupled human muscarinic acetylcholine receptor M1 (CHRM1). Stably transfected monoclonal cell lines were isolated by selection with G418 in 384-well plates. In order to screen the clones for a functional agonist response, 10,000 cells were seeded into white 384-well plates and were left to adhere over night. The next day the medium was discarded and cells from each clone were stimulated with agonist for 1 hour while identical cells in a control well were left untreated.

Comparison of fresh and frozen cells

Cells were prepared according to a standard freezing protocol and used in the assay as follows: After thawing, the cells were washed once in culture medium and resuspended in IP1 stimulation buffer. To compensate for the preincubation time of the fresh cells, which grew over night, the frozen cells were seeded at double density of 20,000 cells per well. The agonist was added to the cells in suspension immediately after seeding.

HTRF® IP-One assay

The IP-One HTRF® assay was performed as recommended by Cisbio. Briefly, IP1-d2 and a Eu-cryptate conjugated antibody against IP1 were added consecutively to the cells. The TR-FRET signals at 665 nm and 620 nm were measured in the PHERAstar microplate reader equipped with a HTRF optical module. The ratio (665 nm/620 nm) was automatically calculated by the MARS data analysis software.

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 and Discussion

Upon stimulation with the physiological ligand acetylcholine (1 µM), recombinant CHO-M1 clones were functionally screened by the IP-One HTRF® assay. (Figure 4)

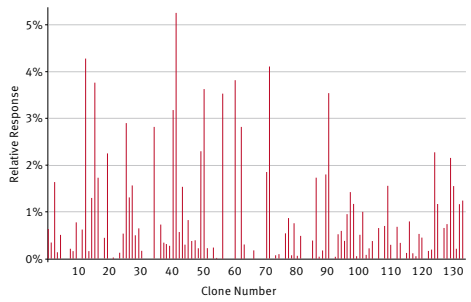

Fig. 4: Screening of single cell clones. Data are displayed in relation to the IP1 standard curve (29 µM IP1 = 100 %).

Out of 134 clones 27 were exhibiting a significant response to the agonist with different sensitivity. One selected cell line (clone B2) was taken to do a dose response curve with carbachol, a partial agonist (Figure 5).

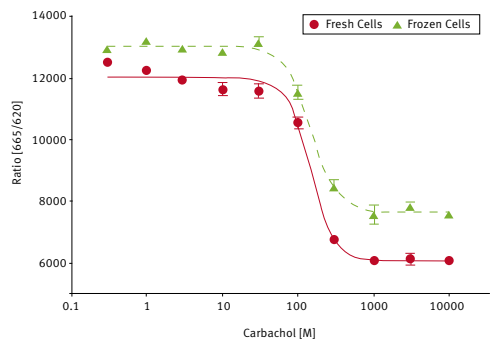

Fig. 5: Dose response of carbachol with cells from a growing culture (fresh cells) and from frozen cells

Although the signal to background ratio was slightly reduced in frozen cells, similar EC_{50} values were determined (Table 1). The Z'factor of the assay with frozen cells was still very good (› 0.7).

Table 1: Comparing data of dose response measurements with Carbachol in fresh and frozen cells

	Fresh Cells	Frozen Cells
Z'factor	0.81	0.77
S/B	2.06	1.69
EC_{50}	147 nM	149 nM

Conclusion

The IP-One HTRF® assay from Cisbio proved to be a fast and reliable method to directly screen a large number of stable trans-fected single cell clones. Strong and medium responding clones expressing a Gαq coupled GPCR could be isolated. One of these selected clones was used to set up a full dose response for agonists. A robust assay could be established using fresh cells from a growing culture as well as Frozen Instant Cells.

References

1 BMG LABTECH Application Note 177:
HTRF® IP-One Terbium-based assay performed on BMG LABTECH's PHERAstar Plus

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