

Cellular and biochemical HTRF® assays measured in 1536-well microplates

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- Two HTRF® assays performed and measured in 1536w format
- PHERAstar® FSX offers robust and fast reading for HTS and compound follow-up
- New TRF laser measures HTRF in a full 1536-well microplate in 36 seconds

Introduction

Screening for new candidate drugs is a standard procedure in the pharmaceutical industry. However, there are increasing requirements on assays and instrumentation. Especially the number of samples measured per day should be further increased. This can be realized if high condensed microplates are used, e.g. 1536-well microplates. The instrument of choice must be able to measure such microplates with the fastest possible read time while the assay performance criteria (such as Z' value or S/B) shall be reached.

To meet the requirements for ultra high throughput screening BMG LABTECH has developed the microplate reader PHERAstar FSX. The new TRF laser enables fastest read times in 1536-well microplates. Two HTRF assays from Cisbio Bioassays were selected to highlight the enhanced performances achieved with the PHERAstar FSX.

Assay Principle

The *HTRF IP-One terbium cellular assay* is the assay of choice to detect levels of myo-Inositol 1 phosphate (IP1) directly in cultured cells. Cells will produce IP1 following a GPCR ligand binding event on a Gq coupled receptor. This assay can be used to study the effect of GPCR agonists and antagonists on the Gq pathway. The assay principle is shown in Fig. 1.

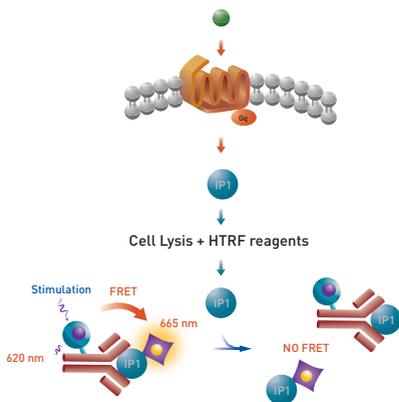


Fig. 1: HTRF® IP-One terbium cellular assay principle.

A monoclonal antibody that is specific for IP1 is labeled with the HTRF donor (Lumi4™-Tb cryptate). Intracellular produced IP1 will compete with HTRF acceptor-labeled

IP1 for the antibody binding sites. The specific HTRF signal is inversely proportional to the concentration of cellular IP1 in the sample.

The second assay detects the interaction of a protein binding domain with a peptide. The assay principle is shown in Fig. 2.

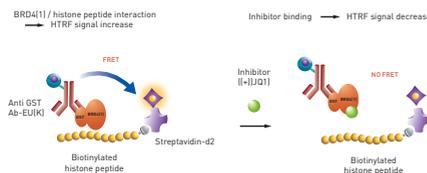


Fig. 2: Histone - BRD4 interaction assay principle.

An anti GST antibody carrying the HTRF donor will bind to a GST tagged bromodomain module (BRD4). A biotinylated histone peptide is bound to the HTRF acceptor. As long as there is interaction between histone peptide and bromodomain a high HTRF signal will be monitored. Inhibitors, such as (+) JQ1, will prevent the interaction leading to a decrease in HTRF signal.

Materials & Methods

- White 1536-well Hi-Base microplates from Greiner
- HTRF IP-One assay and EPIgeneous™ Histone/BRD4 Binding assay from Cisbio
- Certus nano liquid dispenser from Gyger
- PHERAstar FSX microplate reader from BMG LABTECH equipped with flash lamp and TRF laser

All cell culturing and pipetting steps are in accordance with Cisbio's recommendations^{1,2}. The Certus liquid handler is used for reagent dispensing into 1536-well microplates. After the necessary incubation time the microplates are read in the PHERAstar FSX using the following instrument settings:

Detection Method:	Time resolved fluorescence (dual emission)
Optic module:	HTRF
Excitation:	337
EmissionA:	665
EmissionB:	620
Integration start:	60 µs
Integration time:	400 µs
Light source:	Flash Lamp or Laser
Ratio multiplier:	10000

Results & Discussion

IP-One cellular assay

Figure 3 shows the concentration dependent effect of atropine on the level of IP-One.

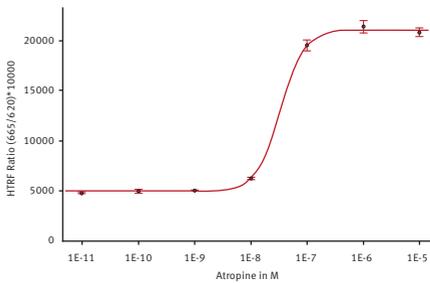


Fig. 3: Antagonist atropine potency determination on CHO-M1 Gq coupled GPCR. Atropine inhibits the effect of a fixed concentration of acetylcholine agonist. Measurement was done on the PHERAstar FSX using the laser in flying mode [1 flash] to measure the HTRF signal. The 4-parameter fit was performed with the MARS Data Analysis software and was weighted using the formula $1/Y^2$.

The measurement was done using the TRF laser in 1 flash flying mode. The 4-parameter standard curve fit shows an R^2 value of 0.9994. From the fit the MARS Data Analysis software calculated the IC_{50} value to be 33 nM which meets our expectations.

Histone-BRD4-Interaction assay

The known inhibitor (+) JQ1 was used to show the inhibitory effect on the histone-BRD4 interaction (Fig. 4).

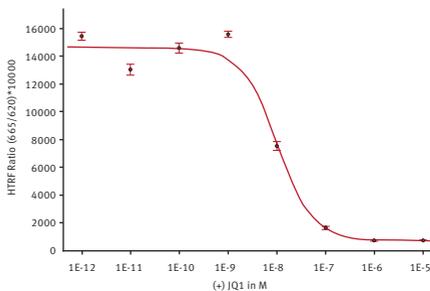


Fig. 4: (+) JQ1 potency determination. Measurement was done on the PHERAstar FSX using the laser in flying mode [1 flash] to measure the HTRF signal. The 4-parameter fit was performed with the MARS Data Analysis software and was weighted using the formula $1/Y^2$.

The above figures show that the HTRF assays can be reliably measured in 1536-well format with the PHERAstar FSX. Using the TRF laser in flying mode for the HTRF measurement will enable the user to read the plate in only 36 seconds while Z' value is excellent. From the data we can also follow that using the laser will lead to higher S/B values compared with flash lamp measurements (Fig. 5).

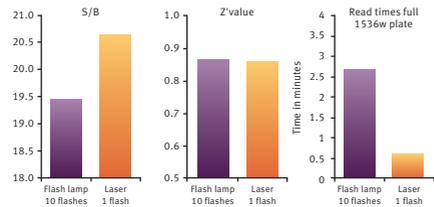


Fig. 5: Histone-BRD4 interaction assay. Comparison of Flash lamp and laser measurements for S/B, Z' value and Read times.

Conclusion

The combination of the HTRF technology from Cisbio with the PHERAstar FSX microplate reader provides reliable tools to fast and reliable high throughput screening and compound follow up.

References

- <http://www.cisbio.com/drug-discovery/ip-one-htrf-assay-kit>
- <http://www.cisbio.com/drug-discovery/epigenoustm-binding-domain-assays>



PHERAstar® FSX