

Implementation of Tag-lite™ Technology on Tecan's Infinite® F500 Multimode Reader

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Abstract

Tag-lite™ is a novel cell surface receptor platform combining SNAP-tag™ and HTRF® technologies for the investigation of cell surface receptors, and G protein-coupled receptor (GPCR) signaling in particular. Tag-lite™ constitutes a complete platform to study ligand binding, receptor dimerization and GPCR-mediated signaling. Transfection of a SNAP-GPCR plasmid into the cell results in the expression of a fully functional GPCR of interest as a fusion protein. The tag can then be labeled with an HTRF® fluorophore.

The present poster summarizes the results of the implementation of the Tag-lite™ technology on Tecan's Infinite F500 filter-based multimode reader, using a cell-based assay to monitor the binding of vasopressin to the V1A receptor, as well as agonist and antagonist competitive inhibition of this ligand. In association with the Infinite F500, Tecan's most sensitive filter-based detection instrument, the Tag-lite™ technology is a powerful tool for studying GPCR mechanistic and signaling.

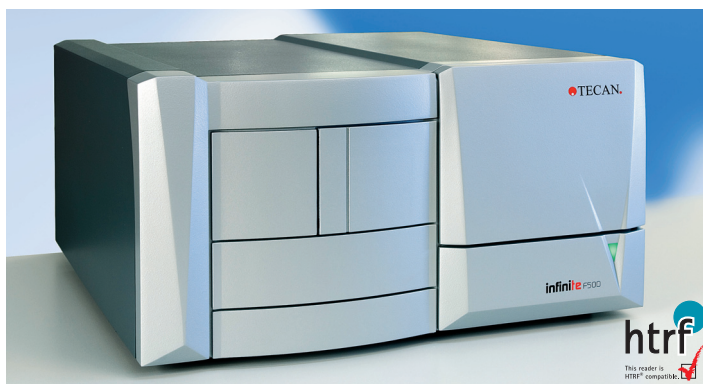


Figure 1: Infinite F500 filter-based multimode reader

Infinite F500

The Infinite F500 is Tecan's most sensitive filter-based detection instrument. It is based on the latest technological developments to provide a multifunctional and modular system that rapidly analyzes all fluorescence-, luminescence- and absorbance based assays at outstanding sensitivity levels.

This flexible new system supports a broad variety of measurement modes, including fluorescence intensity top (UV/VIS) and fluorescence intensity bottom (VIS or UV/VIS); absorbance; luminescence (flash and glow); fluorescence polarization (FP); fluorescence resonance energy transfer (FRET), dual color luminescence; time resolved fluorescence (TRF) and TR-FRET-based assays [Ref 2].

Introduction

HTRF® (homogeneous time-resolved fluorescence) combines time-resolved fluorescence (TRF) with fluorescence resonance energy transfer (FRET) [Ref 1]. It is based on the energy transfer between two fluorophores, a long-lived europium or terbium cryptate donor and either a chemically modified allophycocyanine (XL665) or a d2 acceptor.

HTRF-based measurement applications have become increasingly popular for the analysis of various molecular interactions and binding studies because of their homogeneity, robustness, sensitivity, and potential for miniaturization.

Recently, a new technological concept has been developed to provide a sophisticated tool for the investigation of molecular interactions at the cell surface, specifically those involving G protein-coupled receptors (GPCRs). The Tag-lite™ surface receptor platform combines HTRF and SNAP-tag™ technologies to create a non-radioactive and cost-efficient

method to study receptor dimerization and ligand binding, both of which are central parts of drug discovery research. SNAP tags are suicide enzymes that can be attached to the C- or N-terminal portion of a target protein, e.g. a GPCR of interest. Transfection of cell lines with a custom-engineered GPCR/SNAP tag plasmid results in the expression of a SNAP-tagged GPCR at the cell surface. Labeling of the tag with an HTRF donor fluorophore allows for interaction with specific substrates that are coupled to suitable HTRF acceptor fluorophores.

Its exceptional performance and sensitivity in the field of time-resolved fluorescence applications makes Tecan's Infinite F500 filter-based multimode reader an ideally suited instrument for HTRF-based applications. This poster summarizes the results of the implementation of the Tag-lite™ technology on Tecan's Infinite F500, using a vasopressin ligand-binding assay as an example.

Material and Methods

The functionality of the Tag-lite™ assay system on the Infinite F500 was tested using a receptor-ligand binding assay with Tag-lite™ SNAP-Lumi®-Tb and a red dye-conjugated ligand acceptor. COS-7 cells were transiently transfected with the SNAP tag-Via receptor. A homogeneous ligand binding assay was performed using a Via ligand labeled with a red fluorescent dye. Cells were pipetted into black 384-well low volume microplates (Greiner Bio-One) at a density of 3x10⁴ cells/well in 20 µl each. Serial dilutions (10 µM to 0.01 µM) of vasopressin and antagonist were prepared. 10 µl labeled ligand and 10 µl vasopressin or 10 µl antagonist, respectively, were added to compete with the labeled ligand and the plate was left to incubate for 2 h 40 min at room temperature. The HTRF readout was performed on the Infinite F500 multimode reader using the measurement settings summarized in table 1.

All measurements have been performed by trained scientists from Cisbio Bioassays.

Measurement settings in i-control:

	Label 1: Donor	Label 2: Acceptor
Plate	GRE384fb.pdfx	GRE384fb.pdfx
Measurement Mode	Fluorescence Intensity Top	Fluorescence Intensity Top
Excitation Filter	320 nm	320 nm
Excitation Bandwidth	25 nm	25 nm
Emission Filter	620 nm	665 nm
Emission Bandwidth	10 nm	8 nm
Number of Flashes	10	10
Mirror	Dichroic 510 (e.g. fluorescein)	Dichroic 510 (e.g. fluorescein)
Gain	optimal	optimal
Lag Time	150 µs	150 µs
Integration Time	500 µs	500 µs
Z Position	Calculated from well	Same as Label 1

Table 1: Optimized instrument settings for HTRF measurements on Infinite F500

Results

As shown in figure 2, the addition of vasopressin or antagonist, respectively, leads to a dose-dependent displacement of the red-labeled ligand, producing the sigmoidal curve that is typical for competitive assay formats. The resulting EC50 values are app. 1.335 e-009 M (vasopressin) and app. 1.09 e-009 M (antagonist). The detailed measurement results are summarized in table 2.

	Vasopressin	Antagonist
Top	8917	9097
Bottom	3733	3291
Log EC50	-8,874	-8,962
Hillslope	-1,704	-1,508
EC50	1,335e-009	1,091e-009

Table 2: Vasopressin / antagonist binding assay

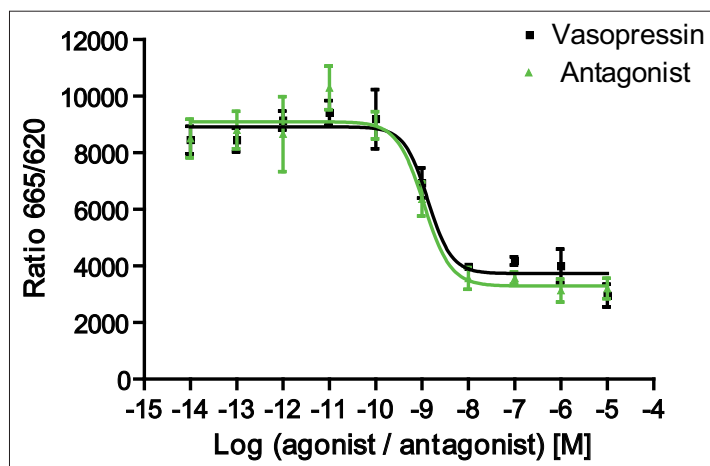


Figure 2: Vasopressin / antagonist binding assay

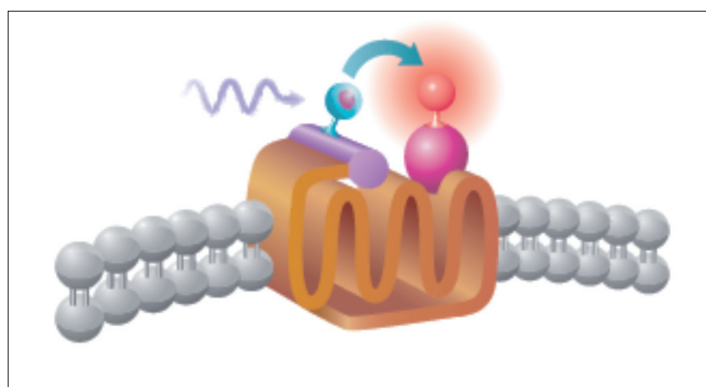


Figure 3: Ligand binding assay using Tag-lite™ SNAP-Lumi4Tb (donor) and a red conjugate ligand (acceptor) [Ref 1]

Conclusion

With the new Tag-Lite™ platform, Cisbio offers a powerful cellular model to monitor events at the cell surface, e.g. ligand binding and functional receptor activation/dimerization. In combination with a highly sensitive multimode reader like the Infinite F500, the Tag-lite™ technology represents a comprehensive platform for high-throughput GPCR analysis. The results summarized in this poster demonstrate that Tecan's Infinite F500 is an excellent detection instrument in terms of sensitivity and reproducibility and, thus, is perfectly compatible with Tag-Lite™-based applications.

References
 [1] <http://www.htrf.com/technology/tag-lite/default.asp>
 [2] www.tecan.com

List of Abbreviations
 COS Cell (cotton) in Origin, carrying the SV40 genetic material
 EC effective concentration
 FRET fluorescence resonance energy transfer
 GPCR G protein-coupled receptor
 HTRF Homogeneous Time-Resolved Fluorescence
 TRF time-resolved fluorescence
 TR-FRET time-resolved resonance energy transfer
 UV/VIS ultraviolet-visible light

www.tecan.com/F500

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