HTRF technology in GPCR and kinase screening

Cisbio international has developed a universal and sensitive assay system that allows for HTS of a large range of GPCRs and kinases, the major targets in drug discovery.

In a recent announcement, Schering AG confirmed that its French subsidiary Schering SAS had completed the transfer of the radiopharmaceutical business of Cisbio international to a consortium formed by the Belgian companies Ion Beam Applications SA (IBA) and the Institut National des Radioéléments (IRE). The new owners have acquired all businesses under the Cisbio international umbrella, including radiopharmaceuticals and in vitro diagnostics.

Cisbio international is an established developer of technologies that are used in assay development and drug screening procedures to enhance drug discovery, and offers a comprehensive technological platform for G-Protein Coupled Receptor Screening (GPCR) screening. Cisbio is a pioneer in the field of homogeneous fluorescence methodologies, and TR-FRET in particular, via its proprietary technology, HTRF®. The company’s HTRF division will continue to operate under the new ownership, and Cisbio says it is committed to R&D initiatives and to partnerships and customer-based programmes where its HTRF technology platform will be effectively deployed.

Targeting GPCRs

GPCRs are transmembrane receptors that transmit signals from the outside to the inside of a cell. They are the most important target class investigated in the drug discovery process. Upon activation, GPCRs carry information within the cell via two major signaling pathways: one results in variations of the cyclic AMP (cAMP) level, whereas the other results in a transient increase of intracellular Ca2+ triggered by inositol(1,4,5)triphosphate (IP3).

GPCRs are understood to be involved in every aspect of human physiology, including sleep regulation, blood pressure, mood, food intake, perception of pain, control of cancer growth and immune response, and are therefore of major interest to pharmaceutical companies because they are druggable targets. Based on this Cisbio recognised the need to develop advanced assay technologies that can more accurately investigate and assess biological mechanisms and provide drug developers with critical information about cell function. Last year, Cisbio launched its homogeneous assay for use in high-throughput drug screening applications, the IP-One Assay System, a screening platform that delivers precise and time-sensitive second messenger measurement, and also represents a novel functional means of precisely investigating GPCR mechanics occurring at the membrane level. IP-One, which is based on Cisbio’s HTRF technology, is the first high throughput system that can easily detect inositol(1)phosphate (IP1), one of the major products of the phosphatidyl inositol cascade, which tightly correlates with Gq-coupled activity.

IP1 is the major degradation product of IP3, inositol(1,4,5)triphosphate. IP-One, the HTRF®-based assay, was developed to quantify the cellular accumulation of IP1, and the design of the assay was enabled through the synthesis of IP1 analogues and by the generation of highly specific monoclonal antibodies directed against IP1.

“Cisbio has consistently proven its commitment to developing critical new assay development technologies for its customers and with our IP-One Assay, we have expanded our capabilities to provide a strategic tool that broadens our customers’ drug development capabilities, specifically in the targeting of GPCRs,” says Francois Degorce, head of HTRF marketing & business development, Cisbio international. “GPCRs are the most important target class investigated in the drug discovery process and the integration of our IP-One assay and Cisbio’s proprietary HTRF technology allows customers to screen these targets with a high degree of reliability. Concurrently, the high pharmacological relevance of the assay produces fine lead characterisation and optimisation that are much sought-after in secondary screening operations or in GPCR mechanistic studies. “This HTRF technology-based assay is homogeneous, non-radioactive, and well adapted to HTS and to miniaturisation,” he adds. “Comparison with reference functional assays such as the quantification of total

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Scientists commenting on HTRF data (from a reader) during an assay development.

HTRF is a highly sensitive, robust technology for the detection of biomolecular interactions and is widely used by the pharmaceutical industry for the high throughput screening stage of drug development. The technology provides a comprehensive technological platform for GPCR screening and kinase screening.

Cisbio international’s IP-One Assay integrates and combines the company’s HTRF technology, allowing for GPCR screening in a high-throughput mode. IP-One incorporates d2, a novel small molecule organic dye developed and optimised by Cisbio and which was also introduced in three cAMP assays due to its advanced properties. This technology provides a technical platform for GPCR investigators to examine both GPCR pathways, and allows HTRF technology to be applied to all GPCR targets. The company has also released a number of specified and ready-to-use assays for the precise quantification of biomarkers such as cytokines, cortisol, PGE2 and most recently, insulin.

The company’s HTRF Toolbox Reagents are used for probing a wide variety of molecular interactions. In recent years, studies have proven the flexibility of this toolbox to address complex cellular mechanisms ranging from nuclear receptor activity to polyubiquitination, as well as heparanase or protease activity.

Cisbio’s HTRF Kinase Toolbox Reagents represent a powerful technology for kinase screening. All HTRF kinase assays use a flexible assay format and allow miniaturisation to 384 low volume or 1536 well formats, and are built around a common cassette system related to the affinity system reagent or the anti-tag toolboxes.

The cassette system comprises the specific anti-phosphoepitope antibody labeled with Eu³⁺ cryptate on one side and on the other, an XL665 conjugate such as Sa-XL⁺⁺ or anti-GST-XL665, available from the company’s affinity system reagent or anti-tag toolboxes, respectively. The HTRF kinase toolbox consists of Eu³⁺-cryptate labeled antibodies for studying both serine/threonine and tyrosine kinases.

**APPLICATIONS OF HTRF TECHNOLOGY**

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**Complementary assay system**

Last month, Cisbio international released its second IP-One assay, IP-One ELISA, complementing the IP-One HTRF assay to create a portfolio of fundamental assays for IP1 (inositol(1)phosphate) quantification and GPCR screening accessible to all laboratories.

IP-One ELISA is a monoclonal antibody-based assay that can easily detect IP1, one of the major products of the phosphatidyl inositol cascade, which tightly correlates with Gq-coupled activity. IP-One ELISA delivers second messenger measurement and represents a way of precisely investigating molecular events occurring at the membrane level. The new kit is an adaptation of the IP-One HTRF to the ELISA detection method.

“Cisbio international continues its commitment to R&D innovation in the field of GPCR screening, and IP-One ELISA is yet another example of this initiative,” says Degorce. “It was important for us to enable a larger customer base, whose resources might not allow for investment in compatible laboratory equipment, to also benefit from IP-One technology.”

**Novel organic dye**

In September of last year, the company launched three new cyclic AMP (cAMP) assays that incorporate d2, a novel small molecule organic dye developed by Cisbio to optimise assay performance and stability when measuring GPCR activation. The upgraded cAMP dynamic 2 and cAMP femto 2 assays, as well as the cAMP HiRange assay, take into account the variability of customers’ biological material and cover a wide range of cAMP concentration. The cAMP dynamic 2 assay is the kit of reference, suitable for most applications, cAMP femto 2 is the most sensitive of the three, and cAMP HiRange is used when the target has high level of expression.

“First introduced in the IP-One Assay, the d2 acceptor molecule has the same photophysical properties as XL665 - the current HTRF acceptor,” says Degorce. “It brings additional benefits to the assays, including a straightforward immunochemistry and negligible compound interference.

Results show extreme consistency and are highly stable over time, so plates can be read for days afterwards, and the assays offer high miniaturisation capabilities. The introduction of the d2-based cAMP assays creates a GPCR screening synergy for Cisbio’s current customers that requires no additional technical resources or know-how.

“The development of the d2-based cAMP assays is testimony to Cisbio’s dedication to new products, as well as to innovative research and development initiatives,” he adds. “d2 is the fruit of our technological research involved in improving HTRF technology for the benefit of our customers, and this acceptor is the cement of all of our assays: after cAMP and IP-One, d2 performance should also benefit other assays in the HTRF product line.”

**FURTHER INFORMATION**

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