



From ELISA to epigenetics with HTRF

HTRF® is a versatile TR-FRET technology developed by Cisbio for detecting biomolecular interactions. Assays encompass the areas of G protein-coupled receptors (GPCR), kinases and cell signaling, epigenetics, and biomarkers.

HTRF detection can be challenging due to the optimization of instrument settings needed to meet assay requirements and detection limits. HTRF assays require a microplate reader with time-resolved fluorescence (TRF) detection mode that is certified HTRF-compatible by Cisbio.

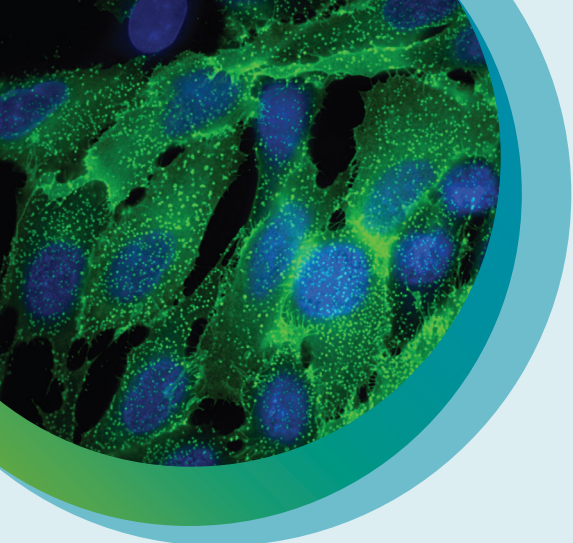
Our SpectraMax® microplate readers are easily configured to be HTRF-ready with dedicated, validated modules to detect HTRF with improved sensitivity. In addition, dedicated HTRF protocols in SoftMax® Pro Software contain pre-optimized settings and built-in calculations that achieve HTRF performance specifications in one click.



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For more information, visit
www.moleculardevices.com



- Homogenous, add-and-read format
- Time and labor saving
- High level of sensitivity
- Amenable to miniaturization
- Application flexibility

Why HTRF?

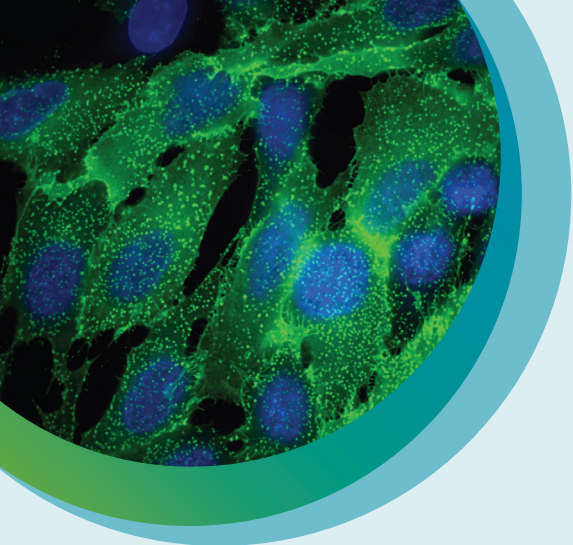
ELISAs (enzyme-linked immunosorbent assays) have helped scientists for over 50 years with their discovery in various fields such as medicine and pharmaceutical research. They are still widely used today as they are inexpensive and easy to set up. However, ELISAs are multi-step assays involving several washing steps and time-consuming incubations. The active agent is detected as absorbance (OD), which can have high background and a limited dynamic range.

To address the limitations of standard ELISAs, new assays have been developed over the past 15+ years including fluorescence-based assays such as time-resolved fluorescence (TRF) and time-resolved fluorescence resonance energy transfer (TR-FRET). These are increasingly used for their ease of use and improved dynamic range.

In particular, the HTRF assay, developed by Cisbio, has many advantages over classical ELISA detection. It detects the interaction of different biological partners, such as dimerizing proteins, two DNA strands, an antigen and an antibody, or a ligand and its receptor. It is a small-volume, homogeneous, no-wash assay following a one-step protocol and offering a high level of sensitivity.

In addition to assays that effectively replace ELISAs, a wide variety of HTRF toolbox reagents and kits is available to address protein-protein interactions, nuclear receptor assays, receptor dimerization, ligand binding, receptor internalization, enzyme assays, and many more.

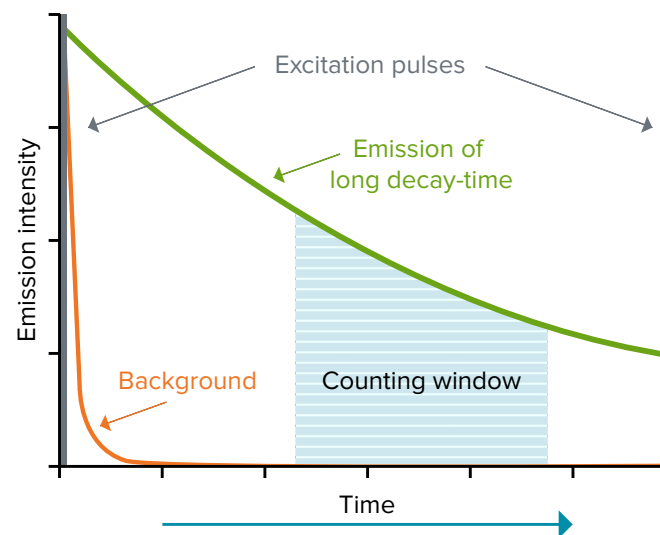
Discover more about TRF, TR-FRET & HTRF [HERE](#) ►



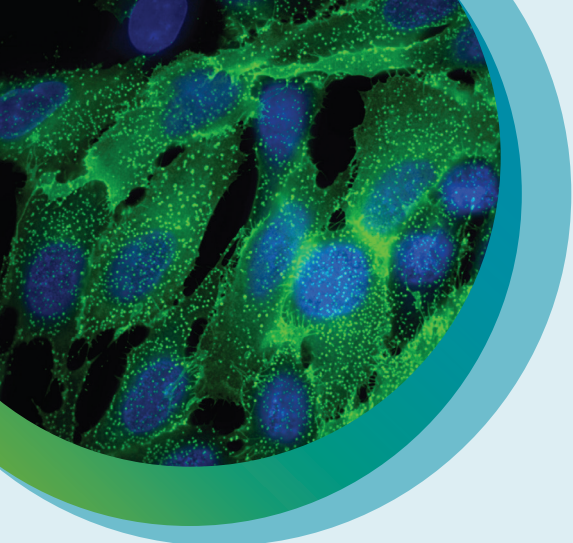
- Low background interference
- Robust
- Rapid, accurate results
- Easy to perform

HTRF assay technology

HTRF involves the use of two fluorophores, a donor and an acceptor, to label proteins or other biomolecules of interest. When the biomolecules interact, their proximity enables fluorescence resonance energy transfer (FRET) to occur from the donor to the acceptor fluorophore. Using long-lifetime fluorescent lanthanide cryptates as donors, allows time-resolved detection of HTRF assays, minimizing short-lived background fluorescence from compounds and other materials. The detection of time-resolved FRET (TR-FRET) with a microplate reader provides a readout of biomolecular binding.



The energy pulse from an excitation source (flash lamp, laser) is immediately followed by a time delay, allowing interfering short-lived fluorescence to decay. Signal is then captured during the 'counting window'.



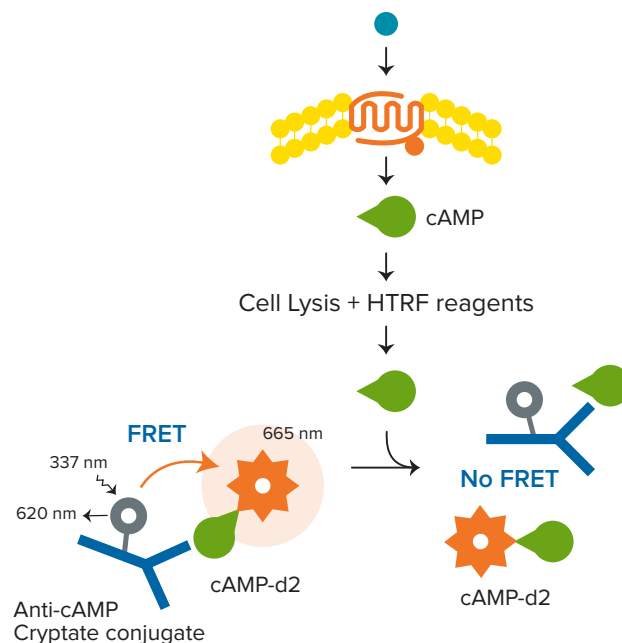
- Wide dynamic range and excellent Z' factors
- Assured instrument performance with certified HTRF compatibility
- Highly robust, homogeneous assays
- Faster time to results with preconfigured SoftMax Pro Software protocols

cAMP assays, $G_{\alpha s}$ -coupled receptors

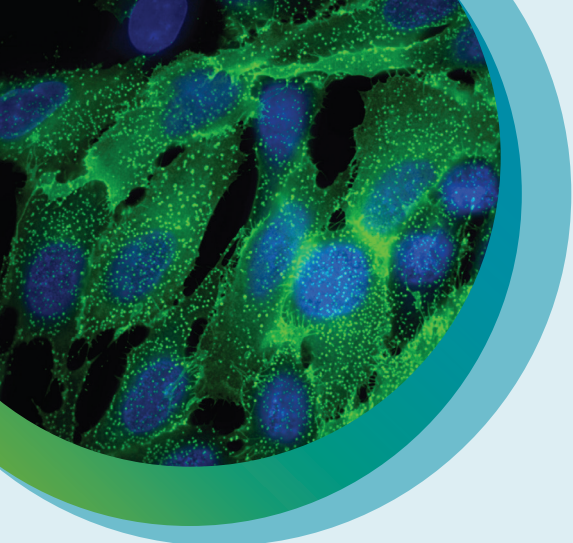
Cyclic AMP (cAMP, cyclic adenosine 3', 5'-monophosphate) is a key second messenger in GPCR signaling. The generation of cAMP is probably the most studied of signal transduction pathways and is involved in responses to sensory input, hormones, nerve transmission, and much more. Upon ligand binding to a GPCR, a conformational change occurs, activating the receptor and in turn activating a G protein. Further signal transduction depends on the type of G protein activated, but activation of $G_{\alpha s}$ leads to upregulation of cAMP by adenylate cyclase.

Explore our resources for $G_{\alpha s}$

- Application Note [HTRF cAMP HiRange Assay on SpectraMax Multi-Mode Microplate Readers](#)
- Application Note [Detect GPCR activity with the cAMP-Gs HiRange HTRF assay](#)
- Poster [HTRF technology on the SpectraMax i3 Multi-Mode Platform](#)
- Poster [Homogeneous High Throughput Live Cell GPCR Functional and Surface Binding Assays](#)



The upregulation of adenylate cyclase results in an increase in cellular cAMP levels. An increase in cellular cAMP leads to a decrease in the FRET signal.



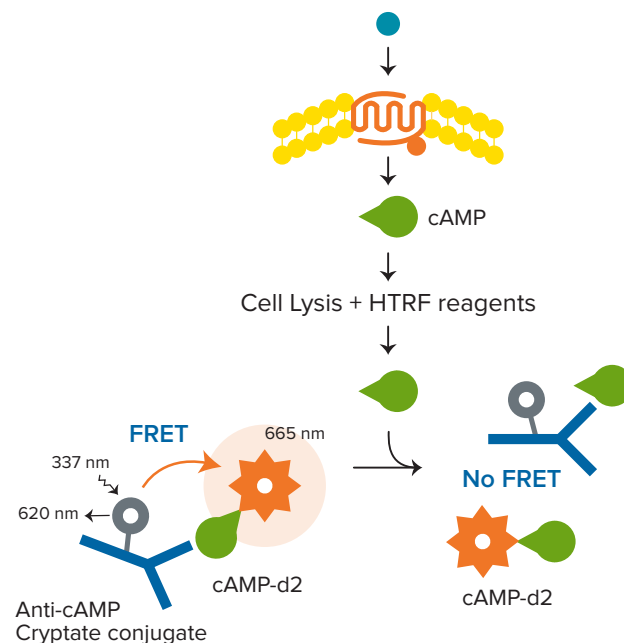
- Wide dynamic range and excellent Z' factors
- Assured instrument performance with certified HTRF compatibility
- Highly robust, homogeneous assays
- Faster time to results with preconfigured SoftMax Pro Software protocols

cAMP assays, $G_{ai/o}$ -coupled receptors

Cyclic AMP (cAMP, cyclic adenosine 3', 5'-monophosphate) is a key second messenger in GPCR signaling. The generation of cAMP is probably the most studied of signal transduction pathways and is involved in responses to sensory input, hormones, nerve transmission, and much more. Upon ligand binding to a GPCR, a conformational change occurs, activating the receptor and in turn activating a G protein. Activation of $G_{ai/o}$ -coupled receptors inhibits adenyl cyclase from generating cAMP.

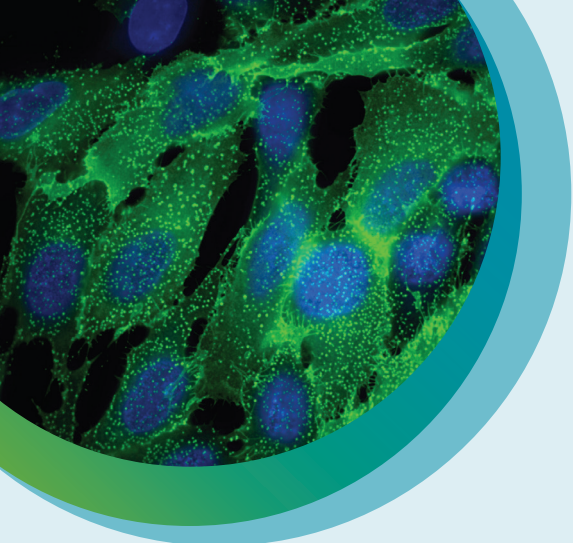
Explore our resources for $G_{ai/o}$

- Application Note [HTRF cAMP dynamic 2 and IP-One assays on the SpectraMax M5e reader](#)
- Poster [HTRF technology on the SpectraMax i3 Multi-Mode Platform](#)
- Poster [Homogeneous High Throughput Live Cell GPCR Functional and Surface Binding Assays](#)



The downregulation of adenylate cyclase results in a decrease in cellular cAMP levels.

A decrease in cellular cAMP leads to an increase in FRET.



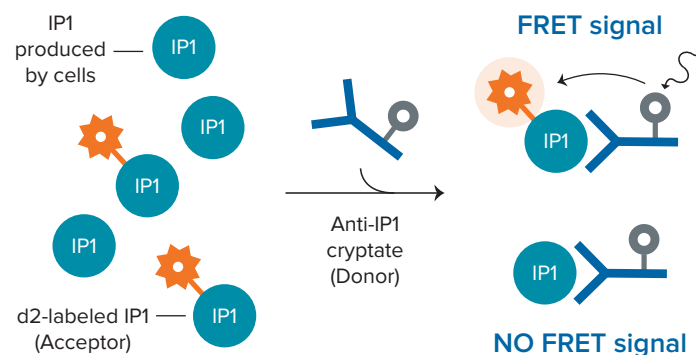
- Wide dynamic range and excellent Z' factors
- Powerful platform for functional assays, suitable for HTS
- Preconfigured HTRF protocols with SoftMax Pro Software; HTRF certified instruments

IP-One assays, $G_{\alpha q/11}$ -coupled receptors

Phospholipase C (PLC) is another common target of activated G proteins and is critical to a variety of processes. For example, thrombin receptors in platelets use this pathway to promote blood clotting. Cisbio IP-One competitive immunoassays detect the accumulation of inositol monophosphate (IP1), a stable downstream metabolite of IP3 induced by the stimulation of PLC following $G_{\alpha q/11}$ -coupled receptor activation. This kit allows direct characterization of receptor agonists and antagonists, in both adherent and suspension cells.

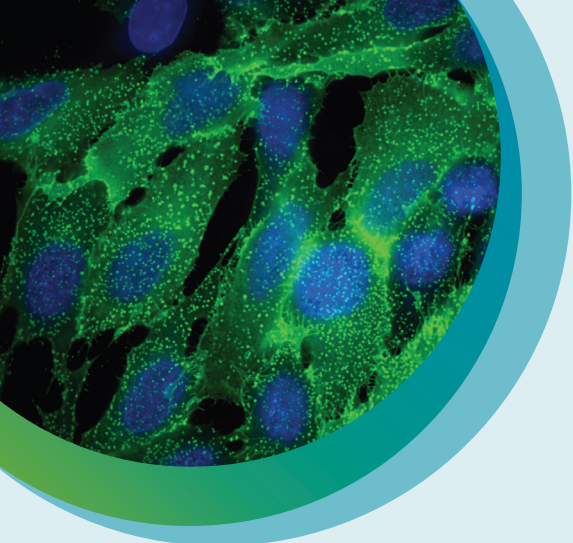
Explore our resources for IP-One assays

- Application Note [HTRF cAMP dynamic 2 and IP-One assays on the SpectraMax M5e Multi-Mode Microplate Reader](#)
- Poster [HTRF technology on the SpectraMax i3 Multi-Mode Microplate Reader](#)



The positive stimulation of phospholipase C (PLC) results in an increase in cellular IP1.

Antagonists prevent the stimulation of PLC, resulting in a decrease in cellular IP1. An increase in cellular IP1 leads to a decrease in FRET.



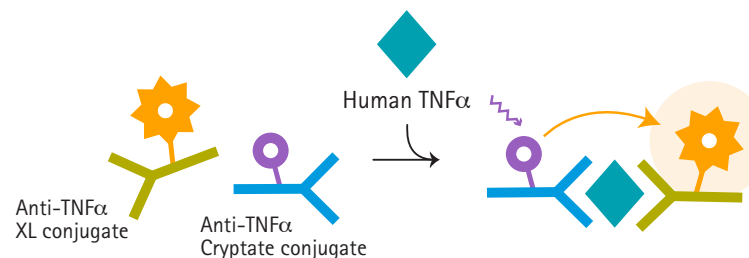
- Evaluate cytokine secretion accurately with sensitive HTRF technology
- Monitor cell viability on the MiniMax cytometer without staining
- Multiplex assays, monitor quality and viability of cells with the EarlyTox™ Cell Integrity Assay Kit

Cytokine assays

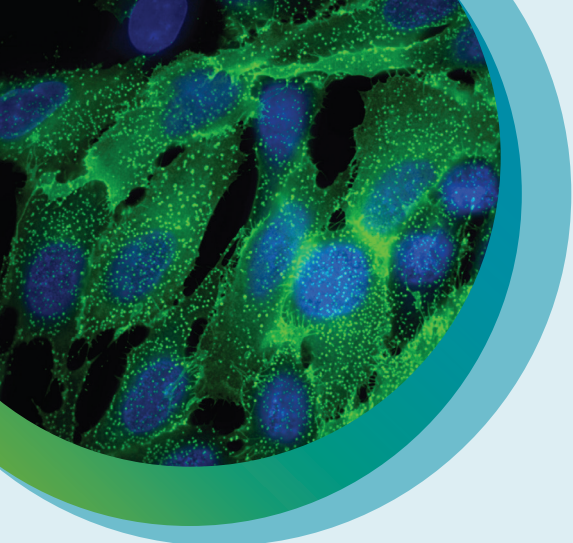
Pro- and anti-inflammatory cytokines play a central role in autoimmune, inflammatory and infectious diseases. They are also key players in metabolic disorders and oncology, particularly the anti-tumor immune response. HTRF assays provide a versatile platform for the quantitation of various cytokines and chemokines and are suited to monitoring cytokine and chemokine release in cell-based experiments. With the SpectraMax i3x Multi-Mode Microplate Reader and SpectraMax® MiniMax™ 300 Imaging Cytometer, users are able to measure HTRF and cell viability, as well as confirm assay quality by monitoring cells' appearance.

Explore our resources for cytokine assays

- Application Note [Normalize HTRF cytokine assays to cell viability](#)
- Application Note [HTRF Human TNFα Assay on SpectraMax Paradigm Multi-Mode Microplate Reader](#)
- eBook [Explore Cells from the Inside Out](#)



Antibodies labeled with donor and acceptor fluorophores bind to TNFα, bringing the labels into close proximity and enabling TR-FRET.



- Universal tool for assessing serine/ threonine and tyrosine kinase activity
- Homogeneous assays save time and money
- Robust technology delivers reliable, reproducible results
- Assured instrument performance with certified HTRF compatibility

Kinase & epigenetic assays

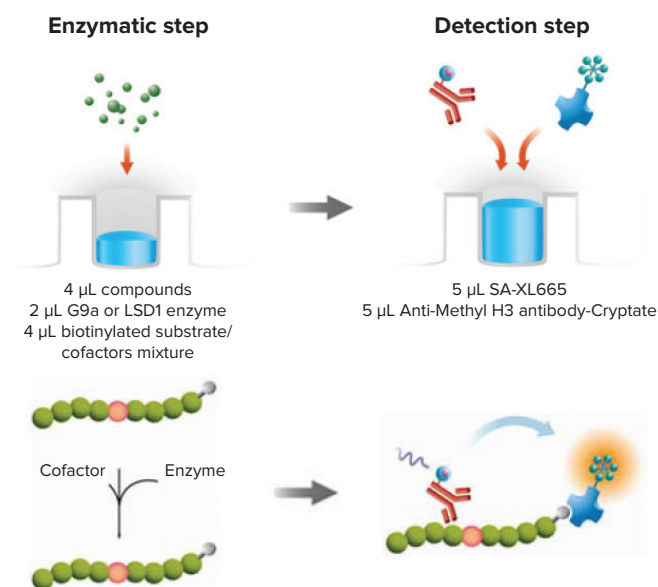
Protein kinases are enzymes that play a central role in various signal transduction pathways involved in the control of cell growth, metabolism, differentiation, and apoptosis. Kinases are the second most important group of drug targets after GPCRs.

Epigenetics is the study of heritable phenotype changes that do not involve alterations in nucleotide sequences, for example, changes such as DNA methylation and histone modification. These have been linked with the development of various diseases, particularly in cancer.

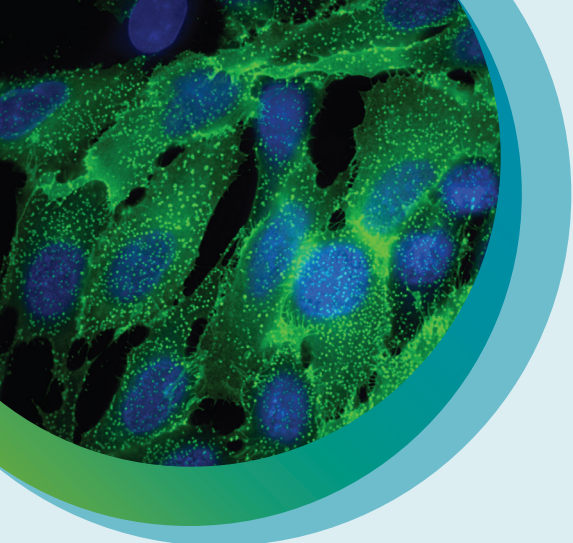
Explore our resources for kinase & epigenetic assays

- Poster [High throughput homogeneous epigenetics assays using HTRF technology and the SpectraMax Paradigm microplate detection platform](#)
- Poster [Homogeneous High Throughput Live Cell GPCR Functional and Surface Binding Assays*](#)

*includes HTRF cellular kinase assay data



Epigenetics assays are run in two main steps, an enzymatic step followed by a detection step using HTRF. In this example, HTRF signal is proportional to the concentration of demethylated peptide.



Molecular Devices HTRF-compatible microplate readers

- SpectraMax® i3x with HTRF Detection Cartridge
- SpectraMax® iD5 with HTRF Detection System
- SpectraMax® Paradigm® with HTRF Detection Cartridge
- SpectraMax® M5e
- FlexStation® 3

Optimized HTRF settings

Getting the best HTRF results requires a microplate reader that has been validated to meet the HTRF performance specifications set by Cisbio. A reader's optics, energy source, and detectors, as well as the settings used to detect HTRF, all impact performance.

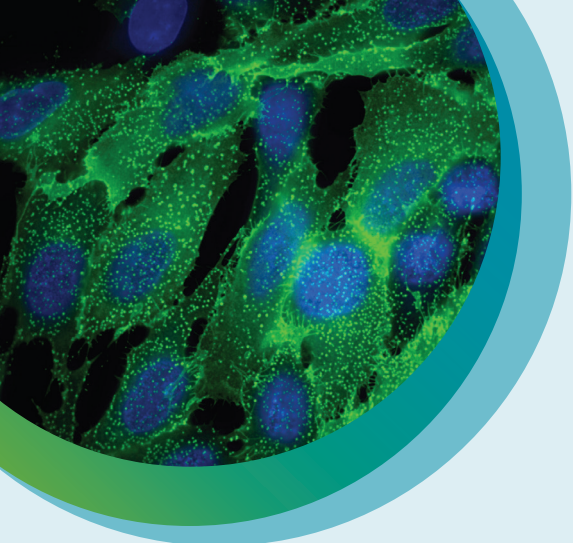
SpectraMax readers use optimized components for HTRF detection, including a HTRF detection cartridge for the SpectraMax i3x and Paradigm Multi-Mode Microplate Readers, and an enhanced TRF module plus validated filters for the SpectraMax iD5 reader.

Explore our resources

- Application Note [Optimized HTRF settings for SpectraMax multi-mode microplate readers](#)
- Application Note [HTRF profiling services on SpectraMax iD5 Multi-Mode Microplate Reader](#)



Cisbio's HTRF certification program guarantees that readers bearing the logo above meet the specifications required for optimal HTRF performance. Molecular Devices HTRF-compatible microplate readers have optimized settings that are fully validated by Cisbio.



- Simplified data measurement
- Flexible data output formats for publication
- Faster time to results with preconfigured SoftMax Pro Software protocols

Simplified HTRF analysis

SoftMax Pro Software contains several preconfigured HTRF protocols to simplify detection and analysis with every HTRF-compatible instrument so you can generate results easily. Analysis of HTRF assays uses Cisbio's patented ratiometric reduction method based on the two emission wavelengths detected. Donor emission at 616 nm is used as an internal reference, while acceptor emission at 665 nm is used as an indicator of the biological reaction being assayed. This ratiometric measurement reduces well-to-well variation and eliminates compound interference. Calculations are built into our HTRF protocols so you can visualize results immediately.

$$1. \text{Ratio} = \frac{\text{Emission}_{665\text{nm}}}{\text{Emission}_{616\text{nm}}} \times 10^4$$

$$2. \text{Mean Ratio} = \frac{\sum \text{ratios}}{2}$$

$$3. \text{CV} = \frac{\text{Std deviation}}{\text{Mean ratio}} \times 100$$

$$4. \text{Delta F} = \frac{\text{Standard or sample Ratio} - \text{Ratio}_{\text{neg}}}{\text{Ratio}_{\text{neg}}} \times 100$$

(Ratio_{neg} = Ratio of negative control)



**SpectraMax i3x Multi-Mode
Microplate Reader**



**SpectraMax iD5 Multi-Mode
Microplate Reader**



**SpectraMax Paradigm
Multi-Mode Microplate
Reader**



**FlexStation 3 Multi-Mode
Microplate Reader**



**SpectraMax M5e
Multi-Mode Microplate
Reader**

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