

# HTRF<sup>®</sup> technology on the SpectraMax<sup>®</sup> i3 Multi-Mode Platform

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## Abstract

HTRF<sup>®</sup> assays have developed over the years to encompass the areas of G protein-coupled receptors, kinases and cell signaling, epigenetics and biomarkers. A wide variety of toolbox reagents address assays including protein-protein interactions, nuclear receptor assays, receptor dimerization, ligand binding, receptor internalization and enzyme assays.

HTRF technology requires specific detection conditions. Time-resolved detection of two different wavelengths compensates for compound interference and sample quenching and also minimizes background fluorescence detection. A plate reader's optics, energy source, and detectors are critical for measuring HTRF output. Instrument set-up also has a significant impact on detection efficiency. Cisbio's certification program guarantees that readers bearing the HTRF sticker meet the physical specifications for optimal HTRF readout.

The new SpectraMax<sup>®</sup> i3 Multi-Mode Platform uses HTRF detection cartridges with optimized components to meet and exceed the specifications required to perform the full range of HTRF assays.



## SpectraMax i3 Multi-Mode Platform

The SpectraMax<sup>®</sup> i3 Multi-Mode Detection Platform comes with standard spectral absorbance, fluorescence, and luminescence detection. Additionally, user-installable options allow the SpectraMax i3 System to address changing application needs and go beyond the standard reader applications. A certified HTRF cartridge enables a full range of HTRF assays from GPCR signaling to epigenetics. The SpectraMax<sup>®</sup> MiniMax<sup>™</sup> Imaging Cytometer option adds cellular imaging capability.

### Key benefits

- Users can upgrade the 3-mode base system with application cartridges for HTRF<sup>®</sup> and other assays, as well as cellular imaging.
- SpectraMax<sup>®</sup> MiniMax<sup>™</sup> Imaging Cytometer simplifies complex imaging workflows.
- SoftMax<sup>®</sup> Pro Microplate Data Acquisition & Analysis Software streamlines complex data analysis with built-in protocols for HTRF and many other applications, with additional tools for regulatory compliance included in SoftMax Pro GxP Software.

## Reader Control Kit

The HTRF Reader Control Kit (P/N 62RCLPEA) is an immunoassay-based kit designed to calibrate HTRF-compatible readers. It was used to determine optimal instrument settings and to demonstrate that the SpectraMax i3 Platform with HTRF cartridge exceeds minimum specifications for HTRF assays using Eu or Tb cryptate and d2 (red) acceptor.

The plate map included a low and a high level calibrator, conjugate blanks, and a specific control to assess the counting rate at 620 nm (i.e. Eu cryptate emission level). The assay was prepared in both white and black plates and incubated >18 hours.

**Table 1.** Results for HTRF Reader Control Kit with black and white microplates. All required specifications were exceeded for both plate types.

Specification	Parameter	Passing	Result (black plate)	Result (white plate)
Standard 0	CV ratio	≤ 10%	6.2%	2.6%
Low calibrator	Delta F %	≥ 15	31.5	29.3
High calibrator	Delta F %	≥ 600	947.4	879.7
Signal/bkg		≥ 40	258.6	215.1

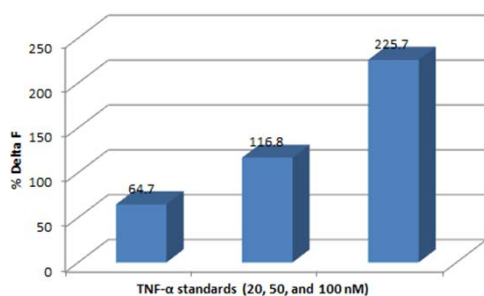
## Optimized Instrument Settings

Instrument setup	
Cartridge	HTRF Detection Cartridge P/N 0200-7011
Number of flashes	30
Integration delay	30 μs
Integration time	400 μs
Optimal read height	Easily optimized for different volumes and plate formats

**Table 2.** Instrument setup for SpectraMax i3 Platform for HTRF assays using Eu or Tb donor with red acceptor.

## TNF-α Assay

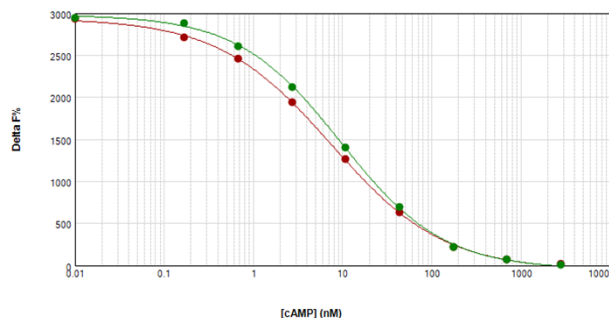
The TNF-α assay was used to evaluate sensitivity of the SpectraMax i3 Platform, with criteria of detection limit and Z' value. Z' values above 0.5 were obtained for calibrator concentrations as low as 50 pM. The detection limit (dose of mean zero + 2SD) was 5 pM, matching the analytical characteristics as stated in the product insert.



**Figure 1.** TNF-α standards at the lower end of the typical assay standard curve. The SpectraMax i3 Platform can detect below the lowest standard, with a calculated detection limit of 5 pM.

## cAMP HiRange Assay

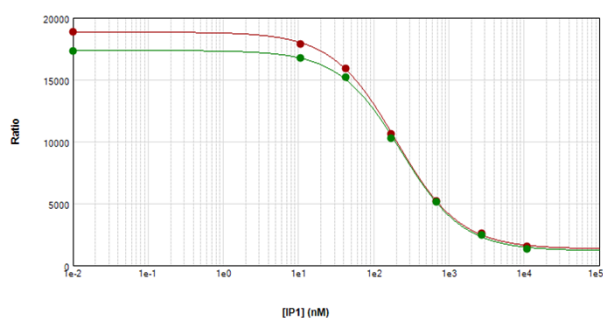
The cAMP HiRange assay kit was used to determine the dynamic range of HTRF on the SpectraMax i3 Platform.



**Figure 2.** cAMP HiRange standard curve in white (red circles) or black (green circles) 384-well microplates. Dynamic range was >2900 (% Delta F). EC<sub>50</sub> values were 7.4 nM and 9.5 nM, respectively, meeting the expected EC<sub>50</sub> of <25 nM in the published product information.

## Ip-One Tb Assay

An Ip-One Tb standard curve was run to assess performance of the SpectraMax i3 Platform for HTRF assays that utilize the Tb donor with red (d2) acceptor.



**Figure 3.** Ip-One Tb standard curve in white (red circles) or black (green circles) 384-well microplates. The EC<sub>50</sub> values, based on final IP1 concentration in the assay, were 137 nM for white plate and 158 nM for black plate, agreeing closely with the published product information.

## Conclusions

The SpectraMax i3 Multi-Mode Platform was fully tested for HTRF performance using Cisbio's standard certification methods. The SpectraMax i3 Multi-Mode Platform was fully tested for HTRF assays using Cisbio's standard certification methods and exhibited excellent performance well exceeding the requirement criteria for HTRF certification. Optimized detection cartridges are available to support the full range of HTRF assays, including Tag-lite.

