

TECHNICAL NOTE

Optimized HTRF settings for SpectraMax multi-mode microplate readers

Caroline Cardonnel | Sr. Applications Scientist | Molecular Devices

HTRF assays from Cisbio have evolved to encompass the areas of G protein-coupled receptors, kinases and cell signaling, epigenetics, and biomarkers. A wide variety of toolbox reagents and kits are available to address protein-protein interactions, nuclear receptor assays, receptor dimerization, ligand binding, receptor internalization, enzyme assays, and many more.

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.

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. Our readers use optimized components for HTRF detection, including an 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.

Cisbio's HTRF certification program guarantees that readers bearing the "HTRF compatible" logo meet the specifications required for optimal HTRF performance. This technical note presents the optimized settings validated by Cisbio for Molecular Devices HTRF-compatible microplate readers.

Molecular Devices HTRF-compatible microplate readers

- SpectraMax® i3x Multi-Mode Microplate Reader
 - HTRF Detection Cartridge (Molecular Devices P/N 0200-7011, or custom TR-FRET for Tb/green)
- SpectraMax® iD5 Multi-Mode Microplate Reader
 - HTRF Detection System (Molecular Devices P/N 6590-0144; includes Enhanced TRF Module, excitation and emission filters)
- SpectraMax® Paradigm® Multi-Mode Microplate Reader
 - HTRF Detection Cartridge (Molecular Devices P/N 0200-7011, or custom TR-FRET for Tb/green)
- SpectraMax® M5e Multi-Mode Microplate Reader
- FlexStation® 3 Multi-Mode Microplate Reader



Figure 1. HTRF-compatible SpectraMax Multi-Mode Readers. SpectraMax i3/i3x and Paradigm readers can be equipped with the HTRF detection cartridge. The SpectraMax iD5 reader uses the HTRF detection system, which includes an Enhanced TRF Module and filters for excitation and emission.

About the reader setup recommendations

HTRF assays are available in three different donor/acceptor fluorophore combinations, each requiring specific reader settings for optimal results:

- Europium cryptate donor / red acceptor (Eu/red)
- Terbium cryptate donor / red acceptor (Tb/red)
- Terbium cryptate donor / green acceptor (Tb/green), Tag-lite

SpectraMax i3/i3x readers

Parameters	Settings	
Europium cryptate donor / red acceptor, Terbium cryptate donor / red acceptor		
Optical configuration	Cartridge	HTRF
Read mode	TR-FRET	
Read type	Endpoint	
Wavelengths	Fixed per cartridge	Ex 340, Em1 616, Em2 665
Plate type	User selectable	White plate required for high-performance HTRF measurement
Read area	User selectable	
PMT and optics	Number of pulses	30
	Excitation time (fixed)	0.05 (fixed)
	Measurement delay	0.03 ms
	Integration time	0.4 ms
	Read height	Plate format- and volume-dependent; optimize for each new plate type or volume used
Terbium cryptate donor / green acceptor, Tag-lite		
Optical configuration	Cartridge	Custom TR-FRET (Tag-lite HTRF)
Read mode	TR-FRET	
Read type	Endpoint	
Wavelengths	Fixed per cartridge	Ex 340/80, Em1 616/15, Em2 665/10
Plate type	User selectable	White plate required for high-performance HTRF measurement
Read area	User selectable	
PMT and optics	Number of pulses	30
	Excitation time (fixed)	0.05 (fixed)
	Measurement delay	0.03 ms
	Integration time	0.4 ms
	Read height	Plate format- and volume-dependent; optimize for each new plate type or volume used

SpectraMax Paradigm reader

Parameters	Settings	
Europium cryptate donor / red acceptor, Terbium cryptate donor / red acceptor		
Cartridge	HTRF	
Read mode	TR-FRET	
Read type	Endpoint	
Wavelengths	Fixed per cartridge	Ex 340, Em1 616, Em2 665
Plate type	User selectable	
Read area	User selectable	
PMT and optics	Number of pulses	30
	Excitation time (fixed)	0.05 (fixed)
	Measurement delay	0.03 ms
	Integration time	0.5 ms
	Read height	Plate format- and volume-dependent; optimize for each new plate type or volume used

Terbium cryptate donor / green acceptor, Tag-lite		
Cartridge	Custom TR-FRET (Tag-lite HTRF)	
Read mode	TR-FRET	
Read type	Endpoint	
Wavelengths	Fixed per cartridge	Ex 340/80, Em1 616/15, Em2 665/10
Plate type	User selectable	
Read area	User selectable	
PMT and optics	Number of pulses	30
	Excitation time (fixed)	0.05 (fixed)
	Measurement delay	0.03 ms
	Integration time	0.5 ms
	Read height	Plate format- and volume-dependent; optimize for each new plate type or volume used

SpectraMax iD5 reader

Parameters	Settings	
Europium cryptate donor / red acceptor, Terbium cryptate donor / red acceptor		
Read mode	TR-FRET	Enhanced TRF module must be installed
Read type	Endpoint	
Wavelengths	Use filters	Ex 340/70 Em1 665/10 Em2 616/10
Plate type	User selectable	
Read area	User selectable	
PMT and optics	Flashes per read	50
	Excitation time (fixed)	0.05 (fixed)
	Measurement delay	0.1 ms
	Integration time	0.6 ms
	Read height	Plate format- and volume-dependent; optimize for each new plate type or volume used

SpectraMax M5e and FlexStation 3 readers

Parameters

Settings

Europium cryptate donor / red acceptor

Read mode	TRF	
Read type	Endpoint	
Wavelengths	Number of wavelength pairs: 2	Ex 314; Em1 620, with 570 cutoff; Em2 665, with 630 cutoff
Plate type	User selectable	White plate required for high performance HTRF measurement
Read area	User selectable	
TRF settings	Integration delay	50
	Integration time	300
PMT and optics	Flashes per read	50 to 100

Terbium cryptate donor / red acceptor

Read mode	TRF	
Read type	Endpoint	
Wavelengths	Number of wavelength pairs: 2	Ex 340; Em1 620, with 570 cutoff; Em2 665, with 630 cutoff
Plate type	User selectable	White plate required for high performance HTRF measurement
Read area	User selectable	
TRF settings	Integration delay	50
	Integration time	300
PMT and optics	Flashes per read	50 to 100

Terbium cryptate donor / Green acceptor, Tag-lite

Read mode	TRF	
Read type	Endpoint	
Wavelengths	Number of wavelength pairs: 2	Ex 340; Em1 520, with 495 cutoff; Em2 620, with 570 cutoff
Plate type	User selectable	White plate required for high performance HTRF measurement
Read area	User selectable	
TRF settings	Integration delay	50
	Integration time	300
PMT and optics	Flashes per read	50 to 100

Conclusion

SpectraMax multi-mode microplate readers with time-resolved fluorescence detection capability were fully tested for HTRF performance by Cisbio using their standard certification methods and exhibited excellent performance while exceeding the requirement criteria for HTRF certification. Optimized detection cartridges or filters are available to support the full range of HTRF assays including Tag-lite.

Contact Us

Phone: +1.800.635.5577
Web: www.moleculardevices.com
Email: info@moldev.com
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