

## HTRF® Europium cryptate donor / Red acceptor readout Setup recommendations for Spark 10M Filter / Filter (FF) configuration

Two sequential measurements should be carried out: at 620 nm for the cryptate emission, and at 665 nm for the specific signal emitted by the acceptor (XL665 or d2). The ratio of the two fluorescence intensities 665/620 (acceptor/donor) enables the calculation of Delta F (%) which represents the relative energy transfer rate for each sample.

The spark 10M must be equipped with the HTRF® module. Spark 10M readers must be appropriately configured for HTRF® readout by setting up the measurement conditions in the Tecan i-Control™ software. In particular, these parameters should be entered as defined in the table below.

### Measurement 1

Excitation filter	320 (25) nm
Emission filter	620 (10) nm
Mirror	Dichroic 510
Lag time	100µs
Integration time	400 µs
Flashes	75
Gain	Optimal gain
Z	Can be calculated on the well giving the highest signal

### Measurement 2

Excitation filter	320 (25) nm
Emission filter	665 (8) nm
Mirror	Dichroic 510
Lag time	100µs
Integration time	400 µs
Flashes	75
Gain	Optimal gain
Z	Can be calculated on the well giving the highest signal



## HTRF® Terbium cryptate donor / Green acceptor readout Setup recommendations for Spark 10M Filter / Filter (FF) configuration

Two sequential measurements should be carried out: at 620 nm for the cryptate emission, and at 520 nm for the specific signal emitted by the acceptor. The ratio of the two fluorescence intensities 520/620 (acceptor/donor) enables the calculation of Delta F (%) which represents the relative energy transfer rate for each sample.

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### Measurement 1

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Excitation filter	340 (35) nm
Emission filter	620 (10) nm
Mirror	Dichroic 510
Lag time	100µs
Integration time	200 µs
Flashes	75
Gain	Optimal gain
Z	Can be calculated on the well giving the highest signal

### Measurement 2

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Excitation filter	340 (35) nm
Emission filter	520 (10) nm
Mirror	Dichroic 510
Lag time	100µs
Integration time	200 µs
Flashes	75
Gain	Optimal gain
Z	Can be calculated on the well giving the highest signal



## HTRF<sup>®</sup> Terbium cryptate donor / Red acceptor readout Setup recommendations for Spark 10M Filter / Filter (FF) configuration

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The spark 10M must be equipped with the HTRF<sup>®</sup> module. Spark 10M readers must be appropriately configured for HTRF<sup>®</sup> readout by setting up the measurement conditions in the Tecan i-Control<sup>™</sup> software. In particular, these parameters should be entered as defined in the table below.

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### Measurement 2

Excitation filter	340 (35) nm
Emission filter	665 (8) nm
Mirror	Dichroic 510
Lag time	100µs
Integration time	200 µs
Flashes	75
Gain	Optimal gain
Z	Can be calculated on the well giving the highest signal



## HTRF® Europium cryptate donor / Red acceptor readout Setup recommendations for Spark 10M Monochromator/ Filter (MF) configuration

Two sequential measurements should be carried out: at 620 nm for the cryptate emission, and at 665 nm for the specific signal emitted by the acceptor (XL665 or d2). The ratio of the two fluorescence intensities 665/620 (acceptor/donor) enables the calculation of Delta F (%) which represents the relative energy transfer rate for each sample.

The spark 10M must be equipped with the HTRF® module. Spark 10M readers must be appropriately configured for HTRF® readout by setting up the measurement conditions in the Tecan i-Control™ software. In particular, these parameters should be entered as defined in the table below.

### Measurement 1

Excitation Monochromator	320 (20) nm
Emission filter	620 (10) nm
Mirror	Dichroic 510
Lag time	100µs
Integration time	400 µs
Flashes	75
Gain	Optimal gain
Z	Can be calculated on the well giving the highest signal

### Measurement 2

Excitation Monochromator	320 (25) nm
Emission filter	665 (8) nm
Mirror	Dichroic 510
Lag time	100µs
Integration time	400 µs
Flashes	75
Gain	Optimal gain
Z	Can be calculated on the well giving the highest signal

**This reader configuration (MF) only allows high performance HTRF measurement when assays are run in WHITE plates.**



## HTRF® Terbium cryptate donor / Green acceptor readout Setup recommendations for Spark 10M Monochromator/ Filter (MF) configuration

Two sequential measurements should be carried out: at 620 nm for the cryptate emission, and at 520nm for the specific signal emitted by the acceptor. The ratio of the two fluorescence intensities 520/620 (acceptor/donor) enables the calculation of Delta F (%) which represents the relative energy transfer rate for each sample.

The spark 10M must be equipped with the HTRF® module. Spark 10M readers must be appropriately configured for HTRF® readout by setting up the measurement conditions in the Tecan i-Control™ software. In particular, these parameters should be entered as defined in the table below.

### Measurement 1

Excitation monochromator	340 (35) nm
Emission filter	620 (10) nm
Mirror	Dichroic 510
Lag time	100µs
Integration time	200 µs
Flashes	75
Gain	Optimal gain
Z	Can be calculated on the well giving the highest signal

### Measurement 2

Excitation Monochromator	340 (35) nm
Emission filter	520 (10) nm
Mirror	Dichroic 510
Lag time	100µs
Integration time	200 µs
Flashes	75
Gain	Optimal gain
Z	Can be calculated on the well giving the highest signal

**This reader configuration (MF) allows high performance HTRF measurement when assays are run in WHITE plates.**



## HTRF® Terbium cryptate donor / Red acceptor readout Setup recommendations for Spark 10M Monochromator/ Filter (MF) configuration

Two sequential measurements should be carried out: at 620 nm for the cryptate emission, and at 665 nm for the specific signal emitted by the acceptor (XL665 or d2). The ratio of the two fluorescence intensities 665/620 (acceptor/donor) enables the calculation of Delta F (%) which represents the relative energy transfer rate for each sample.

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