

## HTRF® Europium cryptate donor / Red acceptor readout Setup recommendations for Spark 20M

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 20M must be equipped with the HTRF® module. Spark 20M 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.

Configuration of head optical for excitation and emission selection				
Configurations : (Excitation/Emission)	Filter/Filter (F/F)	Filter/Monochromator (F/M)	Monochromator/Filter (M/F)	Monochromator/Monochromator (M/M)
<b>Excitation wavelength</b>	320nm/25nm	320nm/25nm	320nm/20nm	320nm/20nm
<b>Acceptor emission wavelength</b>	665nm/8nm	665nm/10nm	665nm/8nm	665nm/10nm
<b>Donor emission wavelength</b>	620nm/10nm	620nm/10nm	620nm/10nm	620nm/10nm
<b>Flashes</b>	50	100	100	100
<b>Lag time</b>	100µs	100µs	100µs	100µs
<b>Integration time</b>	300µs	300µs	300µs	300µs
<b>Mirror</b>	510 dichroic	510 dichroic	510 dichroic	510 dichroic
<b>Gain</b>	Optimal gain	Optimal gain	Optimal gain	Optimal gain
<b>Z optimization</b>	Calculated from well giving the highest signal			
<b>Plate color</b>	Black and white	Black and white	Black and white	<b>White plate only</b>



## HTRF<sup>®</sup> Terbium cryptate donor / Green acceptor readout Setup recommendations for Spark 20M

Two sequential measurements should be carried out at 620nm for the cryptate emission, and at 520nm for the specific signal emitted by the green 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 20M must be equipped with the HTRF<sup>®</sup> module. Spark 20M 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.

Configuration of head optical for excitation and emission selection				
Configurations : (Excitation/Emission)	Filter/Filter (F/F)	Filter/Monochromator (F/M)	Monochromator/Filter (M/F)	Monochromator/Monochromator (M/M)
Excitation wavelength	340nm/35nm	340nm/35nm	340nm/25nm	340nm/25nm
Acceptor emission wavelength	520nm/10nm	520nm/10nm	520nm/10nm	520nm/10nm
Donor emission wavelength	620nm/10nm	620nm/10nm	620nm/10nm	620nm/10nm
Flashes	50	100	100	100
Lag time	100µs	100µs	100µs	100µs
Integration time	300µs	300µs	300µs	300µs
Mirror	510 dichroic	510 dichroic	510 dichroic	510 dichroic
Gain	Optimal gain	Optimal gain	Optimal gain	Optimal gain
Z optimization	Calculated from well giving the highest signal			
Plate color	Black and white	Black and white	Black and white	<b>White plate only</b>



## HTRF® Terbium cryptate donor / Red acceptor readout Setup recommendations for Spark 20M

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 20M must be equipped with the HTRF® module. Spark 20M 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.

Configuration of head optical for excitation and emission selection				
Configurations : (Excitation/Emission)	Filter/Filter (F/F)	Filter/Monochromator (F/M)	Monochromator/Filter (M/F)	Monochromator/Monochromator (M/M)
Excitation wavelength	340nm/35nm	340nm/35nm	340nm/25nm	340nm/25nm
Acceptor emission wavelength	665nm/8nm	665nm/10nm	665nm/8nm	665nm/10nm
Donor emission wavelength	620nm/10nm	620nm/10nm	620nm/10nm	620nm/10nm
Flashes	50	100	100	100
Lag time	100µs	100µs	100µs	100µs
Integration time	300µs	300µs	300µs	300µs
Mirror	510 dichroic	510 dichroic	510 dichroic	510 dichroic
Gain	Optimal gain	Optimal gain	Optimal gain	Optimal gain
Z optimization	Calculated from well giving the highest signal			
Plate color	Black and white	Black and white	Black and white	<b>White plate only</b>

