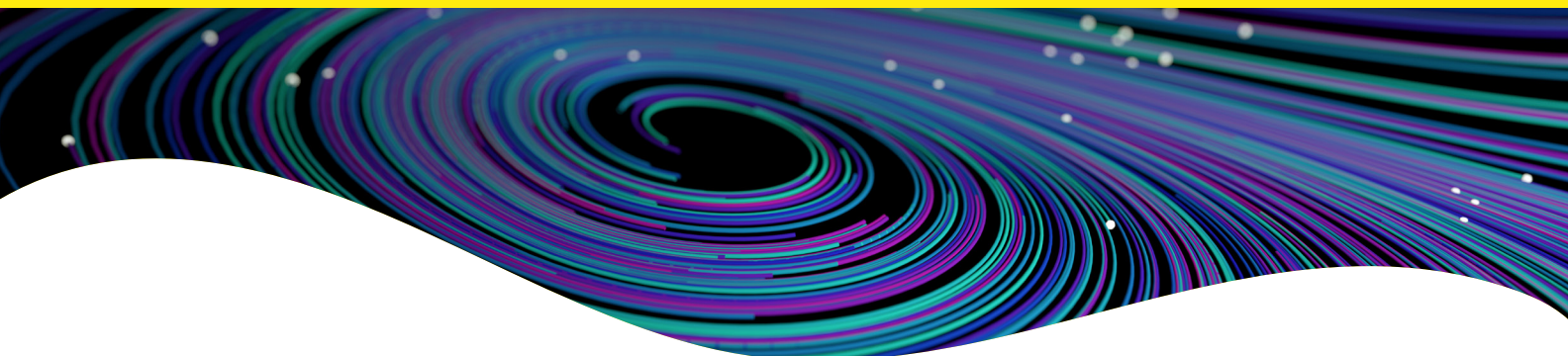# revvity

# HTRF setup recommendations for Spark 20M.

## 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.

| Configuations : (Excitation/ Emission) | Configuration of head optical for excitation and emission selection | | | |
|---|---|---|---|---|
| | Filter/Filter (F/F) | Filter/Monochromator (F/M) | Monochromator/Filter (M/F) | Monochromator/Monochromator (M/M) |
| Excitation wavelength | 320 nm/25 nm | 320 nm/25 nm | 320 nm/20 nm | 320 nm/20 nm |
| Acceptor emission wavelength | 665 nm/8 nm | 665 nm/10 nm | 665 nm/8 nm | 665 nm/10 nm |
| Donor emission wavelength | 620 nm/10 nm | 620 nm/10 nm | 620 nm/10 nm | 620 nm/10 nm |
| Flashes | 50 | 100 | 100 | 100 |
| Lag time | 100 µs | 100 µs | 100 µs | 100 µs |
| Integration time | 300 µs | 300 µs | 300 µs | 300 µs |
| 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 | **White plate only** |

## HTRF 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 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.

| Configuations : (Excitation/ Emission) | Configuration of head optical for excitation and emission selection | | | |
|---|---|---|---|---|
| | Filter/Filter (F/F) | Filter/Monochromator (F/M) | Monochromator/Filter (M/F) | Monochromator/Monochromator (M/M) |
| Excitation wavelength | 340 nm/35 nm | 340 nm/35 nm | 340 nm/25 nm | 340 nm/25 nm |
| Acceptor emission wavelength | 520 nm/10 nm | 520 nm/10 nm | 520 nm/10 nm | 520 nm/10 nm |
| Donor emission wavelength | 620 nm/10 nm | 620 nm/10 nm | 620 nm/10 nm | 620 nm/10 nm |
| Flashes | 50 | 100 | 100 | 100 |
| Lag time | 100 µs | 100 µs | 100 µs | 100 µs |
| Integration time | 300 µs | 300 µs | 300 µs | 300 µs |
| 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 | **White plate only** |

## 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.

| Configuations : (Excitation/ Emission) | Configuration of head optical for excitation and emission selection | | | |
|---|---|---|---|---|
| | Filter/Filter (F/F) | Filter/Monochromator (F/M) | Monochromator/Filter (M/F) | Monochromator/Monochromator (M/M) |
| Excitation wavelength | 340 nm/35 nm | 340 nm/35 nm | 340 nm/25 nm | 340 nm/25 nm |
| Acceptor emission wavelength | 665 nm/8 nm | 665 nm/10 nm | 665 nm/8 nm | 665 nm/10 nm |
| Donor emission wavelength | 620 nm/10 nm | 620 nm/10 nm | 620 nm/10 nm | 620 nm/10 nm |
| Flashes | 50 | 100 | 100 | 100 |
| Lag time | 100 µs | 100 µs | 100 µs | 100 µs |
| Integration time | 300 µs | 300 µs | 300 µs | 300 µs |
| 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 | **White plate only** |