

## HTRF® Europium cryptate donor / Red acceptor readout Setup recommendations for Safire<sup>2</sup>™

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.

Safire<sup>2</sup>™ readers must be appropriately configured for HTRF® readout by setting up the measurement conditions in the "multilabeling" function of Xfluo4 or Magellan software. In particular, these parameters should be entered as below. No special upgrade is required for HTRF® readout, as it is a monochromator-based instrument:

### Measurement 1

|                       |         |
|-----------------------|---------|
| Excitation wavelength | 317 nm  |
| Excitation bandwidth  | 20 nm   |
| Emission wavelength   | 620 nm  |
| Emission bandwidth    | 10 nm   |
| Number of reads       | 100     |
| Lag time              | 60 µs   |
| Integration time      | 500 µs  |
| Gain                  | Optimal |
| Z position            | Optimal |

### Measurement 2

|                       |         |
|-----------------------|---------|
| Excitation wavelength | 317 nm  |
| Excitation bandwidth  | 20 nm   |
| Emission wavelength   | 665 nm  |
| Emission bandwidth    | 10 nm   |
| Number of reads       | 100     |
| Lag time              | 60 µs   |
| Integration time      | 500 µs  |
| Gain                  | Optimal |
| Z position            | Optimal |

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



## HTRF® Terbium cryptate donor / Green acceptor readout Setup recommendations for Safire<sup>2</sup>™

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.

Safire<sup>2</sup>™ readers must be appropriately configured for HTRF® readout by setting up the measurement conditions in the "multilabeling" function of Xfluo4 or Magellan software. In particular, these parameters should be entered as below. No special upgrade is required for HTRF® readout, as it is a monochromator-based instrument:

### Measurement 1

|                       |         |
|-----------------------|---------|
| Excitation wavelength | 343 nm  |
| Excitation bandwidth  | 20 nm   |
| Emission wavelength   | 620 nm  |
| Emission bandwidth    | 10 nm   |
| Number of reads       | 100     |
| Lag time              | 60 µs   |
| Integration time      | 500 µs  |
| Gain                  | Optimal |
| Z position            | Optimal |

### Measurement 2

|                       |         |
|-----------------------|---------|
| Excitation wavelength | 343 nm  |
| Excitation bandwidth  | 20 nm   |
| Emission wavelength   | 520 nm  |
| Emission bandwidth    | 10 nm   |
| Number of reads       | 100     |
| Lag time              | 60 µs   |
| Integration time      | 500 µs  |
| Gain                  | Optimal |
| Z position            | Optimal |

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



## HTRF<sup>®</sup> Terbium cryptate donor / Red acceptor readout Setup recommendations for Safire<sup>2™</sup>

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.

Safire<sup>2™</sup> readers must be appropriately configured for HTRF<sup>®</sup> readout by setting up the measurement conditions in the "multilabeling" function of Xflour4 or Magellan software. In particular, these parameters should be entered as below. No special upgrade is required for HTRF<sup>®</sup> readout, as it is a monochromator-based instrument:

### Measurement 1

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|                       |         |
|-----------------------|---------|
| Excitation wavelength | 340 nm  |
| Excitation bandwidth  | 20 nm   |
| Emission wavelength   | 620 nm  |
| Emission bandwidth    | 10 nm   |
| Number of reads       | 100     |
| Lag time              | 60 µs   |
| Integration time      | 500 µs  |
| Gain                  | Optimal |
| Z position            | Optimal |

### Measurement 2

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|                       |         |
|-----------------------|---------|
| Excitation wavelength | 340 nm  |
| Excitation bandwidth  | 20 nm   |
| Emission wavelength   | 665 nm  |
| Emission bandwidth    | 10 nm   |
| Number of reads       | 100     |
| Lag time              | 60 µs   |
| Integration time      | 500 µs  |
| Gain                  | Optimal |
| Z position            | Optimal |

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

