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HTRF® Europium cryptate donor / Red acceptor readout Setup recommendations for Synergy 4

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 Synergy 4 must be equipped with a TRF module, i.e. SLFTA, or SLFTAD or custom equipped with a T module (TRF).

Synergy 4 readers must be appropriately configured for HTRF® readout by setting up the measurement conditions in the Gen5™ Reader Control and Data Analysis Software. In particular, these parameters should be entered as defined in the table below.

HTRF® assays must be read using the filter-based detection mode only. The monochromator mode is **not** HTRF® compatible.

Measurement 1

| Excitation filter | 330 (80) nm | Ref.: 7082263 |
|--|---|--|
| Emission filter | 620 (10) nm | Ref.: 7082265 |
| Optics position | top 365 | Ref.: 7138365 |
| Number of flashes | 10 | |
| Lag time | 100 µs | |
| Integration time | 300 µs | |
| Sensitivity | Read the plate, select the well with the highest signal, and set its value at 50,000 counts | |
| Z | Select the default value given in the software | |
| Measurement 2 | | |
| | 330 (80) nm | Ref.: 7082263 |
| Excitation filter | 330 (00) 1111 | 110 1002200 |
| Excitation filter Emission filter | 665 (8) nm | Ref.: 7082266 |
| | ` , | |
| Emission filter | 665 (8) nm | Ref.: 7082266 |
| Emission filter Optics position | 665 (8) nm top 365 | Ref.: 7082266 |
| Emission filter Optics position Number of flashes | 665 (8) nm top 365 10 | Ref.: 7082266 |
| Emission filter Optics position Number of flashes Lag time | 665 (8) nm top 365 10 100 μs 300 μs | Ref.: 7082266 Ref.: 7138365 vell with the highest signal, and |

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





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HTRF® Terbium cryptate donor / Green acceptor readout Setup recommendations for Synergy 4

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 fluorescence intensities 520/620 (acceptor/donor) enables the calculation of Delta F (%) which represents the relative energy transfer rate for each sample.

The Synergy 4 must be equipped with a TRF module, i.e. SLFTA, or SLFTAD or custom equipped with a T module (TRF).

Synergy 4 readers must be appropriately configured for HTRF[®] readout by setting up the measurement conditions in the Gen5[™] Reader Control and Data Analysis Software. In particular, these parameters should be entered as defined in the table below.

HTRF[®] assays must be read using the filter-based detection mode only. The monochromator mode is **not** HTRF[®] compatible.

Measurement 1

| Excitation filter | 340 (30) nm | Ref.: 7082230 |
|--|---|---|
| Emission filter | 620 (10) nm | Ref.: 7082265 |
| Optics position | top 400 | Ref.: 7138400 |
| Number of flashes | 10 | |
| Lag time | 100 µs | |
| Integration time | 300 µs | |
| Sensitivity | Read the plate, select the well with the highest signal, and set its value at 50,000 counts | |
| Z | Select the default value given in the software | |
| Measurement 2 | | |
| | | |
| Excitation filter | 340 (30) nm | Ref.: 7082230 |
| | 340 (30) nm 520 (10) nm | Ref.: 7082230 Ref.: 7092163 |
| Excitation filter | ` , | |
| Excitation filter Emission filter | 520 (10) nm | Ref.: 7092163 |
| Excitation filter Emission filter Optics position | 520 (10) nm top 400 | Ref.: 7092163 |
| Excitation filter Emission filter Optics position Number of flashes | 520 (10) nm top 400 10 | Ref.: 7092163 |
| Excitation filter Emission filter Optics position Number of flashes Lag time | 520 (10) nm top 400 10 100 μs 300 μs | Ref.: 7092163 Ref.: 7138400 rell with the highest signal, and |

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HTRF® Terbium cryptate donor / Red acceptor readout Setup recommendations for Synergy 4

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Measurement 1

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|--|---|---|
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| | | |
| Excitation filter | 340 (30) nm | Ref.: 7082230 |
| Excitation filter Emission filter | 340 (30) nm 665 (10) nm | Ref.: 7082230 Ref.: 7082266 |
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