

# HTRF setup recommendations for Synergy 4.

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

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

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

## HTRF Terbium cryptate donor / red acceptor readout setup recommendations for Synergy 4

Two sequential measurements should be carried out: at 620 nm for the cryptate mission, and at 665 nm for the specific signal emitted by the acceptor (XL665 or d2). The ratio of the 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	Measurement 2
Excitation filter	340 (30) nm Ref.: 7082230	340 (30) nm Ref.: 7082230
Emission filter	620 (10) nm Ref.: 7082265	665 (10) nm Ref.: 7082266
Optics position	top 400 Ref.: 7138400	top 400 Ref.: 7138400
Number of flashes	10	10
Lag time	100 µs	100 µs
Integration time	300 µs	300 µs
Sensitivity	Read the plate, select the well with the highest signal, and set its value at 50,000 counts	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	Select the default value given in the software

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

