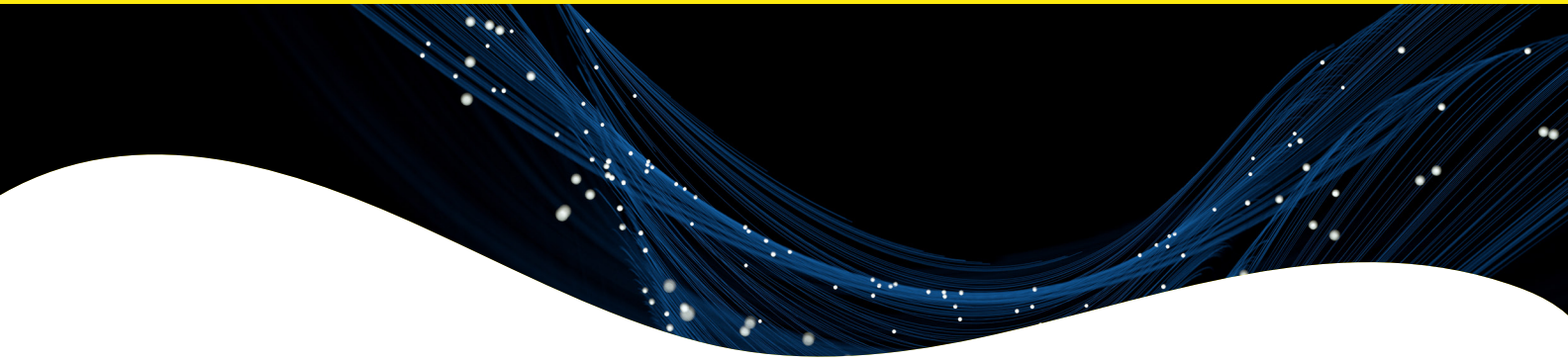
HTRF setup recommendations for Synergy H1.



HTRF Europium cryptate donor / red acceptor readout Setup recommendations for Synergy H1

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 two fluorescence intensities 665/620 (acceptor/donor) enables the calculation of Delta F (%) which represents the relative energy transfer rate for each sample.

Synergy H1 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.

Filter set:
Reference filter cube: 804505

Settings	Filter set 1	Filter set 2
Excitation wavelength Emission wavelength Dichroic mirror	330/80 620/10 365	330/80 665/8 365
Gain	Autoscale	
Read speed	Normal then click on “edit” Delay after plate movement: 100msec Measurement per data point: 10 Lamp energy: High (more sensitivity)	
Time resolved	Click on “edit ” Delay time: 150 µsec Data collection time: 500 µs	
Read height	For first optimization set the gain at 100 then apply the defined value for reading	

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 H1

Two sequential measurements should be carried out: at 620 nm for the cryptate mission, and at 520 nm 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.

Synergy H1 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.

Filter set:

Reference empty block 8040566. The block has to be filled with filters indicated below:

Settings	Filter set 1	Filter set 2
Excitation wavelength Emission wavelength Dichroic mirror	330/80 620/10 365	330/80 520/10 365
Gain	Autoscale	
Read speed	Normal then click on “edit” Delay after plate movement: 100msec Measurement per data point: 10 Lamp energy: High (more sensitivity)	
Time resolved	Click on “edit “ Delay time: 150 µsec Data collection time: 500 µs	
Read height	For first optimization set the gain at 100 then apply the defined value for reading	

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 H1

Two sequential measurements should be carried out: at 620 nm for the cryptate mission, and at 665nm 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.

Synergy H1 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.

Filter set:

Reference filter cube : 804505

Settings	Filter set 1	Filter set 2
Excitation wavelength Emission wavelength Dichroic mirror	330/80 620/10 365	330/80 665/8 365
Gain	Autoscale	
Read speed	Normal then click on “edit” Delay after plate movement: 100msec Measurement per data point: 10 Lamp energy: High (more sensitivity)	
Time resolved	Click on “edit “ Delay time: 150µsec Data collection time: 500µs	
Read height	For first optimization set the gain at 100 then apply the defined value for reading	

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