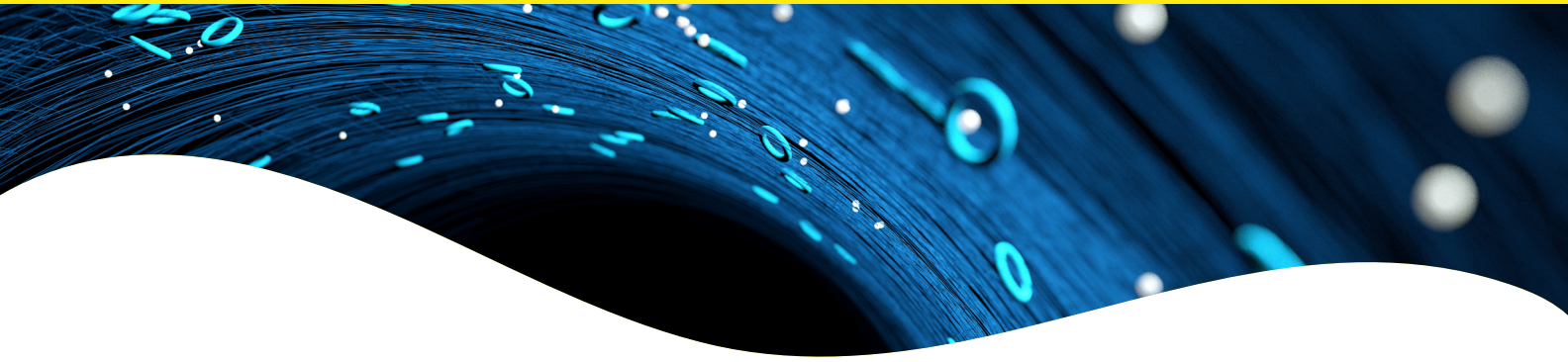
HTRF setup recommendations for Analyst AD/HT.



HTRF Europium cryptate donor / red acceptor readout setup recommendations for Analyst AD/HT

Install the appropriate filter set to read HTRF™ on Analyst® AD/HT excitation by placing the two emission filters next to each other. The Molecular Devices part number for Analyst AD/HT HTRF compatible filter set is 42-000-0063. HTRF method definition under CriterionHost can be carried out as follows:

Define two different FRET reading methods in the TRF dialog box (i.e. one for 620 nm emission and another for 665 nm emission) following the typical settings given below:

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

Main dialog box	665 nm method	620 nm method
Method name	HTRF 665 nm	HTRF 620 nm
Optics	Top	Top
Filters/excitation	330 (80) nm	330 (80) nm
Filters/emission	665 (10) nm	620 (10) nm
Dichroic mirror	BBUV	BBUV
Timing/lashes per well	100	100
Timing/integration time	400 µs	400 µs
Timing/interval between flashes	10 ms	10 ms
Timing/delay after flash	50 µs	50 µs
Z height	e.g. 2 mm	e.g. 2 mm
Raw data units	Counts	Counts
Attenuator mode	Out	Out
PMT setup	Digital	Digital

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

2. Define a reading process in the Multi-Method dialog box (successive 665 nm and 620 nm plate reading)

Name	HTRF readout
Mode/method 1	TRF/665 nm method
Mode/method 2	TRF/620 nm method
Method switching*	By plate

* Well by well counting will decrease throughput but might be of interest for assays requiring more precision

HTRF Terbium cryptate donor / green acceptor readout setup recommendations for Analyst AD/HT

Install the appropriate filter set to read HTRF on Analyst AD/HT excitation by placing the two emission filters next to each other. The Molecular Devices part number for Analyst AD/HT HTRF compatible filter set is 42-000-0063. HTRF method definition under CriterionHost can be carried out as follows:

Define two different FRET reading methods in the TRF dialog box (i.e. one for 620 nm emission and another for 520 nm emission) following the typical settings given below:

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

Main dialog box	520 nm method	620 nm method
Method name	HTRF Tb 520 nm	HTRF Tb 620 nm
Optics	Top	Top
Filters excitation	330 (80) nm	330 (80) nm
Filters/emission	520 (10) nm	620 (10) nm
Dichroic mirror	BBUV	BBUV
Timing/flashes per well	100	100
Timing/integration time	400 µs	400 µs
Timing/interval between flashes	10 ms	10 ms
Timing/delay after flash	50 µs	50 µs
Z height	e.g. 2 mm	e.g. 2 mm
Raw data units	Counts	Counts
Attenuator mode	Out	Out
PMT setup	Digital	Digital

2. Define a reading process in the Multi-Method dialog box (successive 620 nm and 520 nm plate reading)

Name	HTRF readout
Mode/method 1	TRF/520 nm method
Mode/ method 2	TRF/620 nm method
Method switching*	By plate

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 Analyst AD/HT

Install the appropriate filter set to read HTRF on Analyst AD/HT excitation by placing the two emission filters next to each other. The Molecular Devices part number for Analyst AD/HT HTRF compatible filter set is 42-000-0063. HTRF method definition under CriterionHost can be carried out as follows:

Define two different FRET reading methods in the TRF dialog box (i.e. one for 620 nm emission and another for 665 nm emission) following the typical settings given below:

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

Main dialog box	665 nm method	620 nm method
Method name	HTRF 665 nm	HTRF 620 nm
Optics	Top	Top
Filters/excitation	330 (80) nm	330 (80) nm
Filters/emission	665 (10) nm	620 (10) nm
Dichroic mirror	BBUV	BBUV
Timing/flashes per well	100	100
Timing/integration time	400 µs	400 µs
Timing/interval between flashes	10 ms	10 ms
Timing/delay after flash	50 µs	50 µs
Z height	e.g. 2 mm	e.g. 2 mm
Raw data units	Counts	Counts
Attenuator mode	Out	Out
PMT setup	Digital	Digital

2. Define a reading process in the Multi-Method dialog box (successive 665 nm and 620 nm plate reading)

Name	HTRF readout
Mode/method 1	TRF/665 nm method
Mode/method 2	TRF/620 nm method
Method switching*	By plate

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