

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	665nm method	620nm 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

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

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



**The fluorescence ratio is a correction method developed by Cisbio Bioassays with an application limited to the use of HTRF® reagents and technology, and for which Cisbio Bioassays has granted a licence to Molecular Devices. The method is covered by the US patent 5,527,684 and its foreign equivalents.*