

## 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	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.