

HTRF[®] readout - Set up recommendations for Analyst[®] AD/HT readers

Please refer to the set up recommendations page for Analyst[®] GT if you have an Analyst GT in your lab. The present document gives the recommendations for Analyst[®] AD/HT only.

In order to reach maximum performance, we recommend the use of white opaque microplates for HTRF[®] assays on Analyst[®] instruments.

Install the appropriate filter set to read HTRF[®] on Analyst[®] AD/HT (i.e. 330-80 nm excitation, BBUV dichroic mirror, 620-7.5 nm and 665-7.5 nm emission), placing the two emission filters in positions 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 in two steps.

1. 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:

Main dialog box:	620 nm method	665 nm method
Method name	HTRF 620 nm	HTRF 665 nm
Optics	Top	Top
Filters/ excitation	330 nm	330 nm
Filters/ emission	620 nm	665 nm
Plate format	Specify plate type	Specify plate type
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 (to be optimized for each plate format/assay volume)	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 (consecutive 620 nm and 665 nm plate reading).

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

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

For more detailed set up recommendations or if you have specific questions regarding your instrument or your assay, please contact your local Cisbio office.

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