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HTRF® Europium cryptate donor / Red acceptor readout
Setup recommendations for Synergy 2

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.
The Synergy 2 must be equipped with a TRF module, i.e. SLFPTA, or SLFPTAD or custom equipped with a T module (TRF).
Synergy 2 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.

Measurement 1

Excitation filter	330 (80) nm	Ref.: 7082263
Emission filter	620 (10) nm	Ref.: 7082266
Optics position	top 365	Ref.: 7138365
Number of flashes	10	
Lag time	100 µs	
Integration time	300 µs	
Sensitivity	Read the plate, select the well with the highest signal, and set its value at 50,000 counts	
Z	Select the default value given in the software	

Measurement 2

Excitation filter	330 (80) nm	Ref.: 7082263
Emission filter	665 (8) nm	Ref.: 7082265
Optics position	top 365	Ref.: 7138365
Number of flashes	10	
Lag time	100 µs	
Integration time	300 µs	
Sensitivity	Read the plate, select the well with the highest signal, and set its value at 50,000 counts	
Z	Select the default value given in the software	

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 BioTek. The method is covered by the US patent 5,527,684 and its foreign equivalents.*