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HTRF® Europium cryptate donor / Red acceptor readout Setup recommendations for SpectraMax iD5®

Two sequential measurements should be carried out: at 620 nm for the cryptate emission, 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.

SpectraMax iD5 is a hybrid monochromator and filter-based and only filter-based coupled with "Enhanced TRF" module is compatible. The measurement conditions should be set up in the SoftMax® Pro software according to the following indications:

Setup	
TRF module	Must be equipped with Enhanced TRF module (cardridge) in replacing of Standard
Detection mode	TRF module
Excitation filter (bandwidth)	Filter module
Acceptor emission filter (bandwidth)	340nm (70nm)
Donor emission filter (bandwidth)	665nm (10nm)
	616nm (10nm)
Number of flashes	
Excitation time	50
	0.05ms (fixed value)
Measurement delay	0.1ms
Integration time	0.6ms
Read height	Volume and plate format dependant.
	Must be optimized before each new configurated measurement using the labware optimization procedure of the software.





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Setup	
TRF module	Must be equipped with Enhanced TRF module (cartridge) in replacing of Standard TRF module
Detection mode	Tra module
Excitation filter (bandwidth)	Filter module
Acceptor emission filter (bandwidth)	340nm (70nm)
Donor emission filter (bandwidth)	665nm (10nm)
Number of flashes	616nm (10nm)
Excitation time	30
	0.05ms (fixed value)
Measurement delay	0.02ms
Integration time	0.2ms
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Read height	Volume and plate format dependant.
	Must be optimized before each new configurated measurement using the labware optimization procedure of the software.

