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HTRF[®] Terbium cryptate donor / Green acceptor readout Setup recommendations for ULTRA[™]

Two sequential measurements should be carried out: at 620 nm for the cryptate emission, and at 520 nm for the specific signal emitted by the acceptor .The ratio* of the two fluorescence intensities 520/620 (acceptor/donor) enables the calculation of Delta F (%) which represents the relative energy transfer rate for each sample.

UltraTM readers must be appropriately configured for HTRF[®] readout by setting up the measurement conditions in the "multilabeling" function of Xfluor4 or Magellan software. In particular, these parameters should be entered as defined in table below following the installation of the Tecan HTRF[®] upgrade kit (Tecan #B122175) on Ultra[™] and Ultra Evolution[™]

Measurement 1	
Excitation filter	340 (20) nm Ref.: 30000405
Emission filter	620 (10) nm Ref.: 30002292
Mirror	Dichroic 2 (FI 96)
Number of flashes	10
Lag time	150 µs
Integration time	500 µs
Gain	Optimal
Z position	Optimal
Measurement 2	
Excitation filter	340 (20) nm Ref.:30000405
Emission filter	520 (10) nm Ref. :30000463
Mirror	Dichroïc 2 (Fl 96)
Number of flashes	
	10
Lag time	10 150 μs
Lag time Integration time	10 150 μs 500 μs
Lag time Integration time Gain	10 150 μs 500 μs Optimal
Lag time Integration time Gain Z position	10 150 μs 500 μs Optimal Optimal



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