

HTRF[®] Terbium cryptate donor / Red acceptor readout Setup recommendations for FlexStation[®] 3

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.

FlexStation[®] 3 readers must be appropriately configured for HTRF[®] readout by setting up the measurement conditions in the SoftMax Pro software. In particular, these parameters should be entered as defined in the table below. No special upgrade is required for HTRF[®] readout, as it is a monochromator-based instrument.

Read mode	
	Time-Resolved Fluorescence (RFUs)
Integration delay	50 µs
Integration	300 µs
	Top read
Wavelengths	
Excitation 1	340 nm
Emission 1	665 nm
Cut off for emission 1	630 nm
Excitation 2	343 nm
Emission 2	620 nm
Cut off for emission 2	570 nm
Sensitivity	
Readings	50 to 100
PMT	Optimal
Column Wavelength	
	Column Priority

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