

HTRF[®] Tb readout - Set up recommendations for FlexStation[®] 3 readers

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). A ratio of the two fluorescence intensities* (acceptor/donor) then allows the calculation of Delta F (%), i.e. the relative energy transfer rate for each data point.

FlexStation[®] 3 integrates Spectramax M5e reader. Spectramax M5e 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	50us
Integration	300us
	Top read

Wavelengths

Excitation1	340nm
Emission 1	668nm
Cut off for emission 1	630nm
Excitation2	340nm
Emission 2	620nm
Cut off for emission 2	570nm

Sensitivity

Readings	50 to 100
PMT	auto

Column Wavelength Priority

Column Priority

WHITE plates only can be used on this reader!

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