

HTRF[®] Tb readout - Set up recommendations for ULTRA[™] 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. 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.

Ultra readers must be appropriately configured for HTRF[®] Tb readout by setting up the measurement conditions in the "multilabeling" function of XFluor4 software. In particular, these parameters should be entered as defined in the table below.

Measurement 1

Ex Filter	340 (35) nm
Em Filter	620 nm
Mirror	Dichroic3 (e.g. FI 96)
Lag time	150 µs
Integration time	500 µs
Number of flashes	10
Optimal gain	
Optimal z-pos	

Measurement 2

Ex Filter	340 (35) nm
Em Filter	665 nm
Mirror	Dichroic3 (eg FI 96)
Lag time	150 µs
Integration time	500 µs
Number of flashes	10
Optimal gain	

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