

HTRF[®] 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 (XL665). 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[®] 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 following the installation of the Tecan HTRF[®] upgrade kit (Tecan #B122175) on Ultra[™] and Ultra Evolution[™].

Measurement 1

Ex Filter	320 nm
Em Filter	620 nm
Mirror	Dichroic2 (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	320 nm
Em Filter	665 nm
Mirror	Dichroic2 (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.