

HTRF[®] readout - Set up recommendations for Infinite[™] F500 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.

Infinite[™] F500 readers must be appropriately configured for HTRF[®] readout by setting up the measurement conditions in the TECAN i-control[™] or Magellan[™] software. In particular, these parameters should be entered as defined in the table below. The Infinite[™] F500 must be equipped with the HTRF[®] option.

Measurement 1

Excitation filter	320 (25) nm
Emission filter	620 (10) nm
Mirror	Dichroic 510 (e.g fluorescein)
Lag time	150 µs
Integration time	500 µs
Number of reads	10
Gain	Optimal
Z	Can be calculated on any well containing the cryptate molecule

Measurement 2

Excitation filter	320 (25) nm
Emission filter	665 (8) nm
Mirror	Dichroic 510 (e.g fluorescein)
Lag time	150 µs
Integration time	500 µs
Number of reads	10
Gain	Optimal
Z	Can be calculated on any well containing the cryptate molecule

* 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.