

## HTRF<sup>®</sup> readout - Set up recommendations for Infinite<sup>®</sup> M1000 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<sup>®</sup> M1000 readers must be appropriately configured for HTRF<sup>®</sup> readout by setting up the measurement conditions in the "multilabeling" function of i-Control software. In particular, these parameters should be entered as below. No special upgrade is required for HTRF<sup>®</sup> readout, as it is a monochromator-based instrument.

### Measurement 1

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**Excitation wavelength** : 317 nm  
**Excitation bandwidth** : 20 nm  
**Emission wavelength** : 620 nm  
**Emission bandwidth** : 10 nm  
**Number of reads** : 100  
**Lag time** : 60 µs  
**Integration time** : 500 µs  
**Optimal Gain**  
**Optimal Z position**

### Measurement 2

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**Excitation wavelength** : 317 nm  
**Excitation bandwidth** : 20 nm  
**Emission wavelength** : 665 nm  
**Emission bandwidth** : 10 nm  
**Number of reads** : 100  
**Lag time** : 60 µs  
**Integration time** : 500 µs  
**Optimal Gain**  
**Optimal Z position**

**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<sup>®</sup> 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.*