

## HTRF<sup>®</sup> Tb readout - Set up recommendations for Safire<sup>2™</sup> 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.

Safire<sup>2™</sup> readers must be appropriately configured for HTRF<sup>®</sup> 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. No special upgrade is required for HTRF<sup>®</sup> Tb readout, as it is a monochromator-based instrument.

### Measurement 1

|                       |        |
|-----------------------|--------|
| Excitation wavelength | 343 nm |
| Excitation bandwidth  | 20 nm  |
| Emission wavelength   | 620 nm |
| Emission bandwidth    | 10 nm  |
| Lag time              | 60 µs  |
| Integration time      | 500 µs |
| Number of reads       | 50     |
| Optimal gain          |        |
| Optimal z-pos         |        |

### Measurement 2

|                       |        |
|-----------------------|--------|
| Excitation wavelength | 343 nm |
| Excitation bandwidth  | 20 nm  |
| Emission wavelength   | 665 nm |
| Emission bandwidth    | 10 nm  |
| Lag time              | 60 µs  |
| Integration time      | 500 µs |
| Number of reads       | 50     |
| Optimal gain          |        |

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