

HTRF[®] Europium cryptate donor / Red acceptor readout

Setup recommendations for SpectraMax[®] M5^e

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). The ratio* of the two fluorescence intensities 665/620 (acceptor/donor) enables the calculation of Delta F (%) which represents the relative energy transfer rate for each sample.

SpectraMax[®] M5e readers must be appropriately configured for HTRF[®] readout by setting up the measurement conditions in the SoftMax Pro software. In particular, these parameters should be entered as defined in the table below. No special upgrade is required for HTRF[®] readout, as it is a monochromator-based instrument.

| Read mode | |
|--------------------------|-----------------------------------|
| | Time-Resolved Fluorescence (RFUs) |
| Integration delay | 50 µs |
| Integration | 400 µs |
| | Top read |
| Wavelengths | |
| Excitation 1 | 314 nm |
| Emission 1 | 665 nm |
| Cut off for emission 1 | 630 nm |
| Excitation 2 | 314 nm |
| Emission 2 | 620 nm |
| Cut off for emission 2 | 570 nm |
| Sensitivity | |
| Readings | 50 to 100 |
| PMT | Optimal |
| Column Wavelength | |
| | Column Priority |

This reader only allows high performance HTRF measurement when assays are run in WHITE plates.



**The fluorescence ratio is a correction method developed by Cisbio Bioassays with an application limited to the use of HTRF[®] reagents and technology, and for which Cisbio Bioassays has granted a licence to BMG LABTECH. The method is covered by the US patent 5,527,684 and its foreign equivalents.*