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HTRF® Tb readout - Set up recommendations for Synergy 4™ 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.

Synergy 4 readers must be appropriately configured for HTRF® Tb readout by setting up the measurement conditions in the Gen5™ Reader Control and Data Analysis Software. In particular, these parameters should be entered as defined in the table below. The Synergy 4 must be equiped with the TRF module.

HTRF assays must be read using the filter-based detection mode only. The monochromator mode is not HTRF compatible.

USE WHITE PLATES ONLY

Measurement 1

Excitation filter	: 340 (30) nm
Emission filter	: 620 (10) nm
Optics Position	: Top 400 nm
Number of Flashes	: 10
Lag time	: 100µs
Integration time	: 300µs
Sensitivity	: Value to optimise on the well having the highest signal in order to reach 50000 counts.
Z	: Take the default value given in the software.

Measurement 2

Excitation filter	: 340 (30) nm
Emission filter	: 665 (8) nm
Optics Position	: Top 400 nm
Number of Flashes	: 10
Lag time	: 100µs
Integration time	: 300µs
Sensitivity	: Value to optimise on the well having the highest signal in order to reach 50000 counts.
Z	: Take the default value given in the software.

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