

HTRF[®] Tb readout - Set up recommendations for Mithras LB940

The Mithras LB940 reader must be equipped with the TR-FRET reading module which includes the necessary optical components for HTRF[®] readout. Two sequential readings at 620 nm and 665nm emission wavelengths are performed. The ratio* of the fluorescence intensities 665/620 (acceptor/donor) enables the calculation of Delta F (%) which represents the relative energy transfer rate for each sample.

The Mithras LB940 operating software comes with preset ready-to-use parameter files for HTRF[®] Tb measurements including the ratio calculation. The recommended settings are defined under the TR-fluorescence protocol as described below.

Measurement 1 (donor)

	For black & white plate	White plate <u>ONLY</u>
Excitation filter	TRF340/26nm (by default)	TRF320 (by default)
Emission filter	620 (TRF)	620 (TRF)
Lamp Energy	100	100
Cycle time	2000 µs	2000µs
Delay time	50 µs	50 µs
Reading time	400 µs	400 µs
Counting time	1s optimal	1s optimal
Operation mode	By plate	By plate

Measurement 2 (acceptor)

	White & Black plate	White plate <u>ONLY</u>
Excitation filter	TRF340/26nm (by default)	TRF320 (by default)
Emission filter	665 (TRF)	665 (TRF)
Lamp Energy	100	100
Cycle time	2000 µs	2000µs
Delay time	50 µs	50 µs
Reading time	400 µs	400 µs
Counting time	1s optimal	1s optimal
Operation mode	By plate	By plate

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