

HTRF[®] 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[®] measurements including the ratio calculation. The recommended settings are defined under the TR-Fluorescence protocol as described below.

Measurement 1 (donor)

Excitation filter	: D320 (40) (by default)
Emission filter	: D620 (TRF)
Lamp Energy	: 100
Cycle time	: 2000 μ s
Delay time	: 50 μ s
Reading time	: 300 μ s
Counting time	: 1 s Optimal
Operation mode	: by plate

Measurement 2

Excitation filter	: D320 (40) (by default)
Emission filter	: D665 (TRF)
Lamp Energy	: 100
Cycle time	: 2000 μ s
Delay time	: 50 μ s
Reading time	: 300 μ s
Counting time	: 1 s Optimal
Operation mode	: 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 Berthold Technologies. The method is covered by the US patent 5,527,684 and its foreign equivalents.