

# HTRF setup recommendations for Tristar 5.



## HTRF Europium cryptate donor / red acceptor readout setup recommendations for Tristar 5

The Tristar 5 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 665 nm 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.

**Caution:** Among 8 pre-configured models of Tristar 5 reader only 3 models (equipped with Red PMT) are compatible and HTRF certified:

- Research FL (ID: 69185-45)
- Research Plus FL (ID: 69185-50)
- Research Performance FL (ID: 69185-25)

ID is indicated on the label on the back of the reader

The Tristar 5 operating software comes with pre-set 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:

TRF Label	Measurement 1	Measurement 2
Use delay before reading	0.2 s	0.2 s
Counting time	1 s	1 s
Use	Filters	Filters
Aperture	Rd 11	Rd 11
Excitation filter	320/40 HTRF Eu cryptate	320/40 HTRF Eu cryptate
Emission filter	620/10 uv	665/7 uv
Excitation optic	Wide Filter 0.45 mm	Wide Filter 0.45 mm
Cycle time	5000 µs	5000 µs
Delay time	100 µs	100 µs
Reading time	400 µs	400 µs
Operation mode	by plate	by plate

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

## HTRF Terbium cryptate donor / green acceptor readout setup recommendations for Tristar 5

The Tristar 5 reader must be equipped with the TR-FRET reading module which includes the necessary optical components for HTRF readout. Two sequential readings at 520 nm and 620 nm emission wavelengths are performed. The ratio of the fluorescence intensities 520/620 (acceptor/donor) enables the calculation of Delta F (%) which represents the relative energy transfer rate for each sample.

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TRF Label	Measurement 1	Measurement 2
Use delay before reading	0.2 s	0.2 s
Counting time	1 s	1 s
Use	Filters	Filters
Aperture	Rd 11	Rd 11
Excitation filter	340/26 HTRF Tb cryptate	320/26 HTRF Tb cryptate
Emission filter	520/10 uv	620/10 uv
Excitation optic	Wide Filter 0.45 mm	Wide Filter 0.45 mm
Cycle time	2000 $\mu$ s	2000 $\mu$ s
Delay time	50 $\mu$ s	50 $\mu$ s
Reading time	400 $\mu$ s	400 $\mu$ s
Operation mode	by plate	by plate

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

## HTRF Terbium cryptate donor / red acceptor readout setup recommendations for Tristar 5

The Tristar 5 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 665 nm 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.

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Reading time	400 $\mu$ s	400 $\mu$ s
Operation mode	by plate	by plate

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

