

HTRF® Terbium cryptate donor / Red acceptor readout Setup recommendations for SpectraMax iD5®

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 iD5 is a hybrid monochromator and filter-based and **only filter-based is compatible**. The measurement conditions should be set up in the SoftMax® Pro software according to the following indications:

Setup

Detection mode	Filter module
Excitation filter (bandwidth)	340nm (70nm)
Acceptor emission filter (bandwidth)	665nm (10nm)
Donor emission filter (bandwidth)	616nm (10nm)
Number of flashes	30
Excitation time	0.05ms (fixed value)
Measurement delay	0.02ms
Integration time	0.2ms
Read height	Volume and plate format dependant. Must be optimized before each new configured measurement using the labware optimization procedure of the software.

