

HTRF® Europium 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 | 50 |
| Excitation time | 0.05ms (fixed value) |
| Measurement delay | 0.1ms |
| Integration time | 0.6ms |
| Read height | Volume and plate format dependant. Must be optimized before each new configured measurement using the labware optimization procedure of the software. |

