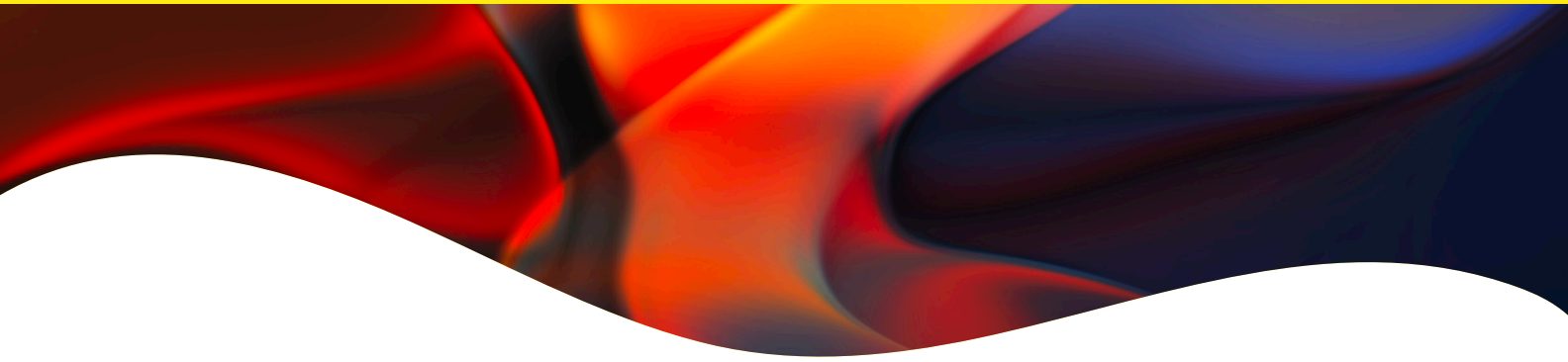
# pHSense™ setup recommendations for Revvity microplate readers.



## pHSense™ readout for EnVision Nexus

EnVision™ Nexus™ is equipped with a specific optical device which enables the measurement of 620nm Europium emission.

pHSense readout can be achieved by EnVision Nexus after the installation of the HTRF/Delfia dedicated optical module which includes the optimizes excitation and emission filters and the dichroic mirror. The measurement conditions should then be set up in the instrument software according to these indications:

| Setup | |
|---|---|
| Measurement mode | Standard |
| Measure from | Top |
| Measurement height | Must be adjusted |
| Measurement time | 800 ms |
| Delay | 1000 µs |
| Window time | 400µs |
| Number of sequential windows | 1 |
| **EnVision Nexus lamp** | |
| Filter module | HTRF™/LANCE (330/665/620) Ref.: HH36794002 – Dual<br>DELFIA (330/615) Ref.: HH36794001 - Single |
| Energy source | Lamp |
| **EnVision Nexus laser** | |
| Filter module | HTRF™/LANCE (343/665/620) Ref.: HH36794003 – Dual<br>DELFIA (615) Ref.: HH36794006 - Single |
| Energy source | Laser |

The pHSense assay operates with a single 620 nm detection mode. If a dual mirror is used, please ignore the 665 nm data.

## pHSense readout for EnVision

EnVision microplate reader must be equipped with specific optical mirror and filters which enable the measurement of 620nm Europium emission.

| Envision lamp Setup | |
|---|---|
| Cycle | 2000 µs |
| Delay | 1000 µs |
| Measurement height | Must be adjusted |
| Number of flash | 100 |
| Total time window | 400 µs |
| **Top mirror dual** | |
| Top mirror | TRF Lance D407/D630 advanced dual mirror (#666) Ref.: 2105-4430<br>TRF LANCE/DELFIA™ D400/D630 dual mirror (#662) Ref.: 2100-4160 |
| Excitation filter | UV2 (TRF) 320 (#111) Ref.: 2100-5060 or<br>UV (TRF) 340 (#101) Ref.: 2100-5010 |
| Emission filter | APC 665 (#205) Ref.: 2100-5110 |
| 2nd emission filter | CY5 620 (#118) Ref.: 2100-5240 or<br>615 (#203) Ref.: 2100-5090 |
| **Top mirror single** | |
| Top mirror | TRF D407 advanced single mirror (#615) Ref.: 2105-4390<br>TRF LANCE/DELFIA D400 single mirror (#412) Ref.: 2100-4170 |
| Excitation filter | UV2 (TRF) 320 (#111) Ref.: 2100-5060 or<br>UV (TRF) 340 (#101) Ref.: 2100-5010 |
| Emission filter | CY5 620 (#118) Ref.: 2100-5240 or 615 (#203) Ref.: 2100-5090 |

EnVision microplate plate reader must be appropriately configured for pHSense readout by setting up the measurement conditions in the software according to these indications:

| Envision laser Setup | |
|---|---|
| Cycle | 16600 µs |
| Delay | 1000 µs |
| Measurement height | Must be adjusted |
| Number of flash | 50 |
| Total time window | 400 µs |
| **Top mirror dual** | |
| Top mirror | TRF LASER D407/D630 advanced dual mirror (#667) Ref.: 2105-4440<br>TRF LASER D400/D630 dual mirror (#446) Ref.: 2103-4290 |
| Emission filter | APC 665 (#205) Ref.: 2100-5110 |
| 2nd emission filter | CY5 620 (#118) Ref.: 2100-5240 or 615 (#203) Ref.: 2100-5090 |
| **Top mirror single** | |
| Top mirror | TRF LASER D407 advanced single mirror (#616) Ref.: 2105-4400<br>TRF LANCE/DELFIA D400 single mirror (#445) Ref.: 2103-4280 |
| Emission filter | CY5 620 (#118) Ref.: 2100-5240 or 615 (#203) Ref.: 2100-5090 |

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

The pHSense assay operates with a single 620 nm detection mode. If a dual mirror is used, please ignore the 665 nm data.

## pHSense readout for VICTOR Nivo

The VICTOR™ Nivo™ must be equipped with specific optical mirror and filters which enable the measurement of 620nm Europium emission.

The VICTOR Nivo reader must be appropriately configured for pHSense readout by setting up the measurement conditions in the software according to these indications:

| Operation | TRF-EndPoint |
|---|---|
| Measurement type | Single label |
| Excitation filter | 320/75 nm Ref.: HH35000947 |
| Emission filter | 620/10 nm Ref.: HH35000936 or 615/8 nm Ref.: HH35000935 |
| Dichroic mirror | D400 Ref.: HH35000971 |
| Delay time (µs) | 1000 |
| Emission time (µs) | 400 |
| Measurement direction | Top |
| Measurement time (ms) | 500 |
| Z-focus (mm) | Must be adjusted |
| Flash energy (mJ) | Low (10) |

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