

Filter overview for fluorescent dyes

Infinite[®] 200 PRO and Spark[®]



The fluorescence filters listed are available for Infinite 200 PRO and Spark multimode readers with filter-based fluorescence optics.

The recommended filter combinations for each dye were selected according to the excitation and emission properties (1) of the dye, and based on a formula to avoid crosstalk (3). Please be aware that excitation and emission properties of a fluorescent dye will vary with assay conditions – such as pH, salt concentrations and labeling partners – and that derivatives of a dye may have altered spectra. Suitability of the filter recommendation for your specific assay cannot be guaranteed, and other filter combinations might also be suitable. Users must always independently evaluate and validate filters for their intended use.

Dye	Dye properties		Excitation filter	Emission filter
	Ex	Em	WL (BW)	WL (BW)
5'-ROX (5-carboxy-X-rhodamine)	578 nm	604 nm	560 nm (20 nm)	610 nm (20 nm)
5-FAM (5-Carboxyfluorescein)	492 nm	518 nm	485 nm (20 nm)	535 nm (25 nm)
AlamarBlue [®] (resazurin/resurofin)	571 nm	583 nm	560 nm (10 nm)	590 nm (20 nm)
Alexa Fluor [®] 532	531 nm	554 nm	520 nm (10 nm)	560 nm (20 nm)
Alexa Fluor 546	556 nm	573 nm	540 nm (25 nm)	590 nm (20 nm)
Alexa Fluor 555	555 nm	565 nm	530 nm (25 nm)	595 nm (35 nm)
Alexa Fluor 647	650 nm	668 nm	620 nm (20 nm)	680 nm (30 nm)
Allophycocyanin (APC)	645 nm	660 nm	620 nm (20 nm)	670 nm (25 nm)
AMC (amino-methylcoumarin), pH 9	360 nm	448 nm	380 nm (20 nm)	430 nm (20 nm) 460 nm (20 nm)
Amplex [®] Red (resorufin)	571 nm	585 nm	540 nm (25 nm)	590 nm (20 nm)
BCECF	503 nm	528 nm	485 nm (20 nm)	535 nm (25 nm)
Bodipy 581/591	584 nm	592 nm	560 nm (20 nm)	625 nm (35 nm)



Dye	Dye properties		Excitation filter	Emission filter
	Ex	Em	WL (BW)	WL (BW)
Bodipy FI	505 nm	513 nm	485 nm (20 nm)	535 nm (25 nm)
Bodipy R6G SE	528 nm	547 nm	505 nm (20 nm)	560 nm (20 nm)
Bodipy TMR	542 nm	570 nm	530 nm (25 nm)	580 nm (20 nm) 595 nm (35 nm)
Bodipy TR	589 nm	617 nm	560 nm (20 nm)	635 nm (35 nm)
Calcein AM	494 nm	517 nm	485 nm (20 nm)	535 nm (25 nm)
Cascade Blue	400 nm	420 nm	380 nm (20 nm)	430 nm (20 nm)
CFP	435 nm	477 nm	430 nm (20 nm)	485 nm (20 nm)
Cy3	554 nm	566 nm	530 nm (25 nm)	580 nm (20 nm) 595 nm (35 nm)
Cy3.5	576 nm	598 nm	560 nm (20 nm)	620 nm (20 nm) 635 nm (35 nm)
Cy5	647 nm	665 nm	620 nm (20 nm)	680 nm (30 nm)
Cy5.5	672 nm	692 nm	635 nm (35 nm)	720 nm (40 nm)
DAPI (DNA)	358 nm	461 nm	360 nm (35 nm)	465 nm (35 nm)
DCFDA	505 nm	525 nm	485 nm (20 nm)	535 nm (25 nm) 540 nm (25 nm)
DELFLIA® (europium)			340 nm (35 nm)	612 nm (10 nm)
DsRed	556 nm	583 nm	530 nm (25nm)	590 nm (20 nm) 595 nm (35 nm)
DsRed	556 nm	583 nm	540 nm (25 nm)	590 nm (20 nm)
eGFP	489 nm	511 nm	485 nm (20 nm)	535 nm (25 nm)
Ethidium homodimer-1 (EthD-1)	528 nm	617 nm	535 nm (25 nm)	620 nm (20 nm) 635 nm (35 nm)
Europium - chelate	365 nm	610 nm	340 nm (20 nm) 340 nm (35 nm)	610 nm (20 nm)
eYFP	514 nm	527 nm	485 nm (20 nm)	535 nm (25 nm)
eYFP	514 nm	527 nm	510 nm (10 nm)	535 nm (10 nm)
FDA (fluoresceindiacetat)	498 nm	516 nm	485 nm (20 nm)	535 nm (25 nm)
FITC (fluorescein-5-isothiocyanate)	495 nm	519 nm	485 nm (20 nm)	535 nm (25 nm)
Fluo-3	506 nm	527 nm	485 nm (20 nm) 495nm (10 nm)	520nm (10 nm) 535nm (10 nm)
Fluorescein	489 nm	517 nm	485 nm (20 nm)	535nm (25 nm)
Fura-2 (<i>Ex changes from 366 nm to 336 nm on Ca²⁺ binding</i>)	366 nm (336 nm)	505 nm	340 nm (20 nm) 380 nm (20 nm)	510 nm (25 nm)
Hoechst® 33258 (DNA)	352 nm	455 nm	360 nm (35 nm)	465 nm (35 nm)
Hoechst 33342	348 nm	455 nm	360 nm (35 nm)	465 nm (35 nm)



Dye	Dye properties		Excitation filter	Emission filter
	Ex	Em	WL (BW)	WL (BW)
HTRF® (Cisbio, europium) <i>both Em filter necessary</i>			320 nm (25 nm)	620 nm (10 nm) 665 nm (8 nm)
HTRF (Cisbio, Terbium) <i>both Em filter necessary</i>			340 nm (35 nm)	620 nm (10 nm) 665 nm (8 nm)
Lissamine™ Rhodamine	570 nm	590 nm	560 nm (10 nm) 560 nm (20 nm)	610 nm (20 nm)
mCherry	587 nm	610 nm	560 nm (20 nm)	620 nm (20 nm)
Monobromobimane (mBBr)	394 nm	490 nm	380 nm (20 nm) 400 nm (20 nm)	465 nm (35 nm) 485 nm (20 nm)
NADH	340 nm	466 nm	340 nm (20 nm)	465 nm (35 nm)
NanoOrange®	470 nm	570 nm	465 nm (35 nm) 485 nm (20 nm)	560 nm (20 nm) 580 nm (20 nm) 580 nm (30 nm)
Phycoerythrin B	545 nm	575 nm	535 nm (10 nm)	580 nm (20 nm) 590 nm (20 nm)
Phycoerythrin R	565 nm	576 nm	540 nm (25 nm)	590 nm (20 nm)
PicoGreen® (DNA)	502 nm	522 nm	485 nm (20 nm)	535 nm (25 nm)
PO-PRO-1	280 nm	457 nm	280 nm (15 nm)	460 nm (10 nm) 460 nm (20 nm)
Propidium iodide (PI)	535 nm	617 nm	535 nm (25 nm)	620 nm (20 nm)
RFP	555 nm	584 nm	535 nm (25 nm)	590 nm (20 nm)
SYBR® Green I (DNA)	498 nm	522 nm	485 nm (20 nm)	535 nm (25 nm)
SYTO™ 17 (DNA)	620 nm	638 nm	610 nm (20 nm)	670 nm (25 nm)
TAMRA	553 nm	576 nm	535 nm (25 nm)	590 nm (10 nm) 590 nm (20 nm)
Terbium - chelate (TR-FRET; LanthaScreen™, Invitrogen)			340 nm (35nm)	495 nm (10 nm)
Texas Red	592 nm	614 nm	560 nm (20 nm)	620 nm (20 nm) 635 nm (35 nm)
Texas Red	592 nm	614 nm	580 nm (20 nm)	620 nm (10 nm)
TMR (Tetramethylrhodamine)	552 nm	578 nm	535 nm (25 nm)	590 nm (20 nm)
TRITC	555 nm	576 nm	535 nm (25 nm) 540 nm (25 nm)	590 nm (20 nm)
Tryptophan	278 nm	354 nm	280 nm (15 nm)	Scanning (300 - 500 nm) for con- formation studies
YFP	513 nm	530 nm	485 nm (20 nm) 495 nm (10 nm)	535 nm (25 nm) 540 nm (25 nm)



How to select a suitable filter pair for a fluorescent dye

- 1) Obtain the excitation and emission maxima from the dye's data sheet, suitable literature or database (eg. https://www.nightsea.com/sfa-sharing/fluorescence-spectra-viewers*).
- 2) Select filters with wavelength properties (WL + BW) covering the excitation or emission maxima.
- 3) Check the filter selection for spectral overlap using the following formula:

$$WL_{EM} - BW_{EM} - WL_{EX} - BW_{EX} = \geq 5 \text{ nm}$$

WL = Wavelength of the filter EX = Excitation
 BW = Bandwidth of the filter EM = Emission

At least 5 nm distance is recommended between the upper end of the spectral range of the excitation filter ($WL_{EX} + BW_{EX}$) and the lower end of the spectral range of the emission filter ($WL_{EM} - BW_{EM}$).

- 4) Evaluate and validate the selected filters for your specific assay and use.

If no suitable filters are available in the list, please contact your local Tecan sales representative.

Example: fluorescein

- Excitation maximum: 489 nm
- Emission maximum: 517 nm
- Recommended excitation filter: 485 nm (20 nm)
- Recommended emission filter: 535 nm (25 nm)

$$535 - 25 - 485 - 20 = \geq 5 \text{ nm}$$

The recommended filters for fluorescein cover the respective maxima (excitation and emission), and show the recommended minimal spectral distance of 5 nm.

Important information:

Instrument measurement specifications are tested and validated for specific dyes and filters only, as stated in the instructions for use for the instrument. Measurement limits may be different for other dyes and filters. Users must always independently evaluate and validate the suitability of filters for their intended use.

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