

## EnVision Filter Application List

Version 1.0

MMD Application Support Team

### How to use this list

Search for the fluorophore or assay label you want to use in one of the lists and find the suggested filter and dichroic mirror combination.

Within the filter name, the central wavelength of the filter is given as first number, followed by “/” and the filter bandwidth in full-width at half maximum (FWHM) and “nm”. For example “480/30nm” denotes a filter with central wavelength at 480nm and a bandwidth of 30nm (FWHM).

A dichroic mirror is denoted by the cut-on wavelength. Wavelength ranges below are reflecting excitation light to the sample, wavelength ranges above are transmitting emission light from the sample to the detector. The use of a dichroic mirror in Fluorescence and Fluorescence polarisation assays is optional, but gives better results than the Standard 50/50 beamsplitter.

**Please note that there is no need for filter central wavelength and the peak wavelengths usually given for fluorophores to be identical!**

### Fluorescence Dye List

Fluorophore	Excitation filter	Emission filter	Mirror module
<b>5-TAMRA</b>	2100-5830 Tamra SNP 531/25 attenuated filter	2100-5600 Tamra, Cy3 579/25 filter	2100-4120 BODIPY TMR D555 single mirror
<b>Alamar Blue</b>	2100-5050 Cy3, Tamra FI/FP 531/25 filter or* 2100-5830 Tamra SNP 531/25 attenuated filter	2100-5500 Photometric 590/8 filter or 2100-5330 Rhodamine 590/20 filter	2100-4120 BODIPY TMR D555 single mirror
<b>Alexa 350</b>	2100-5030 Umbelliferone 355/40 filter	2100-5130 Umbelliferone 460/25 filter	2100-4170 LANCE/DELTA D400 single mirror
<b>Alexa 488</b>	2100-5020 FITC 485/14 filter	2100-5120 FITC 535/25 filter	2100-4030 FITC D505 single mirror
<b>Alexa 555</b>	2100-5050 Cy3, Tamra FI/FP 531/25 filter	2100-5600 Tamra, Cy3 579/25 filter	2100-4120 BODIPY TMR D555 single mirror
<b>Alexa 568</b>	2100-5670 Texas Red FP 555/39	2100-4360 Texas Red module	2100-5590 Emission 635/15
<b>Alexa 594</b>	2100-5670 Texas Red FP 555/38	2100-5590 Emission 635/15	2100-4360 Texas Red module

Fluorophore	Excitation filter	Emission filter	Mirror module
<b>Alexa 647</b>	2100-5240 Cy5 620/10 filter	2100-5770 Cy5 685/35 filter	2100-4240 Cy5 D658 single mirror
<b>AMC (7-Amino-4-Methylcoumarin)</b>	2100-5030 Umbelliferone 355/40 filter	2100-5130 Umbelliferone 460/25 filter	2100-4170 LANCE/DELFLIA D400 single mirror
<b>Amplex Red</b>	2100-5050 Cy3, Tamra FI/FP 531/25 filter	2100-5530 Emission 595/60 filter	2100-4120 BODIPY TMR D555 single mirror
<b>APC (Allophycocyanin)</b>	2100-5240 Cy5 620/10 filter	2100-5770 Cy5 685/35 filter	2100-4240 Cy5 D658 single mirror
<b>Blue Fluorescent Protein (BFP)</b>	2100-5030 Umbelliferone 355/40 filter	2100-5130 Umbelliferone 460/25 filter	2100-4170 LANCE/DELFLIA D400 single mirror
<b>Bodipy-TMR</b>	2100-5050 Cy3, Tamra FI/FP 531/25 filter	2100-5530 Emission 595/60 filter	2100-4120 BODIPY TMR D555 single mirror
<b>Calcein</b>	2100-5020 FITC 485/14 filter	2100-5120 FITC 535/25 filter	2100-4030 FITC D505 SINGLE MIRROR
<b>Cerulean</b>	2100-5840 CFP 430/24 filter	2100-5850 CFP 470/24 filter	2100-4310 CFP/YFP D450_515 single mirror
<b>Cyan Fluorescent Protein (CFP)</b>	2100-5840 CFP 430/24 filter	2100-5850 CFP 470/24 filter	2100-4310 CFP/YFP D450_515 single mirror
<b>Citrine</b>	2100-5840 CFP 430/24 filter	2100-5850 CFP 470/24 filter	2100-4310 CFP/YFP D450_515 single mirror
<b>Cy3</b>	2100-5050 Cy3, Tamra FI/FP 531/25 filter	2100-5600 Tamra, Cy3 579/25 filter	2100-4120 BODIPY TMR D555 single mirror
<b>Cy5</b>	2100-5240 Cy5 620/10 filter	2100-5770 Cy5 685/35 filter	2100-4240 Cy5 D658 single mirror
<b>DAPI</b>	2100-5030 Umbelliferone 355/40 filter	2100-5130 Umbelliferone 460/25 filter	2100-4170 LANCE/DELFLIA D400 single mirror
<b>dsRED</b>	2100-5050 Cy3, Tamra FI/FP 531/25 filter	2100-5760 Cy5 620/40 filter	2100-4360 Texas Red module
<b>DyLight 488</b>	2100-5020 FITC 485/14 filter	2100-5120 FITC 535/25 filter	2100-4030 FITC D505 single mirror
<b>FAM</b>	2100-5020 FITC 485/14 filter	2100-5120 FITC 535/25 filter	2100-4030 FITC D505 single mirror
<b>FITC</b>	2100-5020 FITC 485/14 filter	2100-5120 FITC 535/25 filter	2100-4030 FITC D505 single mirror

Fluorophore	Excitation filter	Emission filter	Mirror module
<b>Fluo-4</b>	2100-5020 FITC 485/14 filter	2100-5120 FITC 535/25 filter	2100-4030 FITC D505 single mirror
<b>Fura-2</b>	2100-5390 Fura2, BFP 380/10 filter 2100-5750 Photometric Fura-2 340/14 filter	2100-5320 Fura2 510/10 filter	2100-4170 LANCE/DELFLIA D400 SINGLE MIRROR
<b>(e)GFP: (enhanced) Green Fluorescent Protein</b>	2100-5020 FITC 485/14 filter	2100-5120 FITC 535/25 filter	2100-4030 FITC D505 single mirror
<b>HEX</b>	2100-5050 Cy3, Tamra FI/FP 531/25 filter	2100-5600 Tamra, Cy3 579/25 filter	2100-4120 BODIPY TMR D555 single mirror
<b>Hoechst 33342</b>	2100-5030 Umbelliferone 355/40 filter	2100-5130 Umbelliferone 460/25 filter	2100-4170 LANCE/DELFLIA D400 SINGLE MIRROR
<b>mCherry</b>	2100-5670 Texas Red FP 555/38	2100-5590 Emission 635/15	2100-4360 Texas Red module
<b>mOrange</b>	2100-5050 Cy3, Tamra FI/FP 531/25 filter	2100-5600 Tamra, Cy3 579/25 filter	2100-4120 BODIPY TMR D555 single mirror
<b>PicoGreen</b>	2100-5020 FITC 485/14 filter	2100-5120 FITC 535/25 filter	2100-4030 FITC D505 SINGLE MIRROR
<b>Red Fluorescent Protein (RFP)</b>	2100-5050 Cy3, Tamra FI/FP 531/25 filter	2100-5600 Tamra, Cy3 579/25 filter	2100-4120 BODIPY TMR D555 single mirror
<b>Resorufin</b>	2100-5050 Cy3, Tamra FI/FP 531/25 filter	2100-5530 Emission 595/60 filter	2100-4120 BODIPY TMR D555 single mirror
<b>Rhodamine 110</b>	2100-5020 FITC 485/14 filter	2100-5120 FITC 535/25 filter	2100-4030 FITC D505 single mirror
<b>ROX</b>	2100-5670 Texas Red FP 555/38	2100-5760 Cy5 620/40 filter	2100-4360 Texas Red module
<b>SybrGreen</b>	2100-5020 FITC 485/14 filter	2100-5120 FITC 535/25 filter	2100-4030 FITC D505 single mirror
<b>Texas Red</b>	2100-5670 Texas Red FP 555/38	2100-5590 Emission 635/15	2100-4360 Texas Red module
<b>Td Tomato</b>	2100-5050 Cy3, Tamra FI/FP 531/25 filter	2100-5600 Tamra, Cy3 579/25 filter	2100-4120 BODIPY TMR D555 single mirror
<b>TRITC</b>	2100-5050 Cy3, Tamra FI/FP 531/25 filter	2100-5600 Tamra, Cy3 579/25 filter	2100-4120 BODIPY TMR D555 single mirror

<b>Fluorophore</b>	<b>Excitation filter</b>	<b>Emission filter</b>	<b>Mirror module</b>
<b>Umbelliferone</b>	2100-5030 Umbelliferone 355/40 filter	2100-5130 Umbelliferone 460/25 filter	2100-4170 LANCE/DELTA D400 single mirror
<b>Venus</b>	2100-5860 YFP 500/20 filter	2100-5870 YFP 530/35 filter	2100-4310 CFP/YFP D450_515 single mirror
<b>Yellow Fluorescent Protein (YFP)</b>	2100-5860 YFP 500/20 filter	2100-5870 YFP 530/35 filter	2100-4310 CFP/YFP D450_515 single mirror

\*Depending on fluorophore concentration, needs to be tested in the assay.

For multicolour assays like Förster Resonance Energy Transfer (FRET) assays, the ideal filter combinations might be different to those listed above. Please contact PerkinElmer for additional support.

For Fluorescence Polarization Assays, the most common filters are available in a special FP version (not listed here).

If a dye is not listed here, there is a good chance it can still be measured with the available filters. In order to find them, use a fluorophore database or ask your assay kit manufacturer to name a spectral analogue which is listed here.

For more information or customized solutions, please refer to your local PerkinElmer contact.

## Time-Resolved Fluorescence (TRF) Dye List

Some assays listed below are dual readout assays where two detection channels are measured. On the EnVision XCite, this is done subsequently while on the EnVision HTS, a simultaneous readout is performed, which requires a different mirror module.

### Standard Series

Assay Type	Excitation	Emission filter	2nd emission filter	Mirror module Xcite	Mirror module HTS
<b>DELFLIA</b>	2100-5010 UV (TRF340) dug11 + wg320 filter	2100-5090 Europium 615/8.5 filter	-	2100-4170 LANCE/DELFLIA D400 single mirror	2100-4170 LANCE/DELFLIA D400 single mirror
<b>LANCE</b>	2100-5060 UV (TRF320) dug11 filter	2100-5110 LANCE (APC, Alexa) 665/7.5 filter	2100-5090 Europium 615/8.5 filter	2100-4170 LANCE/DELFLIA D400 single mirror	2100-4160 LANCE/DELFLIA D400/630 dual mirror
	TRF Laser	2100-5110 LANCE (APC, Alexa) 665/7.5 filter	2100-5090 Europium 615/8.5 filter or* 2105-5980 LANCE Laser attenuated Europium Filter	2103-4280 TRF LASER D400 single mirror	2103-4290 TRF LASER D400/D630 dual mirror
<b>homogeneous TRF</b>	2100-5060 UV (TRF320) dug11 filter	2100-5110 LANCE (APC, Alexa) 665/7.5 filter	2100-5240 Cy5 620/10 filter	2100-4170 LANCE/DELFLIA D400 single mirror	2100-4160 LANCE/DELFLIA D400/630 dual mirror
	TRF Laser	2100-5110 LANCE (APC, Alexa) 665/7.5 filter	2100-5240 Cy5 620/10 filter	2103-4280 TRF LASER D400 single mirror	2103-4290 TRF LASER D400/D630 dual mirror
<b>LanthaScreen</b>	2100-5060 UV (TRF320) dug11 filter	2100-5880 TRF Emission 520/25 filter	2100-5890 TRF Emission 495/10 filter	2100-4170 LANCE/DELFLIA D400 single mirror	2100-4300 TRF D400/D505 dual mirror
	TRF Laser	2100-5880 TRF Emission 520/25 filter 2100	-5890 TRF Emission 495/10 filter	2100-4170 LANCE/DELFLIA D400 single mirror	2103-4340 TRF LASER D400/D505 dual mirror

\*: for LANCE assays with particularly bright emission of Europium

### Advanced Series

Choose the sets from this list for an improved TRF performance.

Assay Type	Excitation	Emission filter	2nd emission filter	Mirror Module Xcite	Mirror Module HTS
<b>DELFI</b>	2100-5010 UV (TRF340) dug11 + wg320 filter	2100-5090 Europium 615/8.5 filter	-	2105-4390 TRF advanced single mirror	2105-4390 TRF advanced single mirror
<b>LANCE</b>	2100-5060 UV (TRF320) dug11 filter	2100-5110 LANCE (APC, Alexa) 665/7.5 filter	2100-5090 Europium 615/8.5 filter	2105-4390 TRF advanced single mirror	2105-4430 TRF LANCE D407/D630 advanced dual mirror
	TRF Laser	2100-5110 LANCE (APC, Alexa) 665/7.5 filter2100	-5090 Europium 615/8.5 filter or* 2105-5980 LANCE Laser attenuated Europium Filter	2105-4400 TRF LASER advanced single mirror	2105-4440 TRF LASER LANCE D407/D630 advanced dual mirror
<b>Homogeneous TRF</b>	2100-5060 UV (TRF320) dug11 filter	2100-5110 LANCE (APC, Alexa) 665/7.5 filter	2100-5240 Cy5 620/10 filter	2105-4390 TRF advanced single mirror	2105-4430 TRF LANCE D407/D630 advanced dual mirror
	TRF Laser	2100-5110 LANCE (APC, Alexa) 665/7.5 filter	2100-5240 Cy5 620/10 filter	2105-4400 TRF LASER advanced single mirror	2105-4440 TRF LASER LANCE D407/D630 advanced dual mirror
<b>LanthaScreen</b>	2100-5060 UV (TRF320) dug11 filter	2100-5880 TRF Emission 520/25 filter2100	-5890 TRF Emission 495/10 filter	2105-4390 TRF advanced single mirror	2105-4410 TRF D407/D505 advanced dual mirror
	TRF Laser	2100-5880 TRF Emission 520/25 filter2100	-5890 TRF Emission 495/10 filter	2105-4400 TRF LASER advanced single mirror	2105-4420 TRF LASER D407/D505 advanced dual mirror

\*: for LANCE assays with particularly bright emission of Europium

## Absorbance Filter List

The table below lists the available filters for absorbance. Check your Assay description which one to use.

<b>Filter Name</b>	<b>Central Wavelength / nm</b>
2100-5340 Photometric 260/10 filter	260
2100-5350 Photometric 280/10 filter	280
2100-5740 Photometric 320/14 filter	320
2100-5200 Photometric 405/8 filter	405
2100-5400 Photometric 420/8 filter	420
2100-5660 Photometric 440/8 filter	440
2100-5210 Photometric 450/8 filter	450
2100-5450 Photometric 475/8 filter	475
2100-5220 Photometric 492/8 filter	492
2100-5460 Photometric 530/8 filter	530
2100-5420 Photometric 540/8 filter	540
2100-5430 Photometric 550/9 filter	550
2100-5470 Photometric 560/8 filter	560
2100-5630 Photometric 570/8 filter	570
2100-5500 Photometric 590/8 filter	590
2100-5560 Photometric 595/10 filter	595
2100-5640 Photometric 600/8 filter	600
2100-5620 Photometric 610/10 filter	610
2100-5650 Photometric 620/8 filter	620
2100-5730 Photometric 720/8 filter	720
2100-5700 Photometric 750/8 filter	750
2100-5750 Photometric, Fura-2 340/14 filter	340

## Luminescence Dye List

Some assays listed below are dual readout assays where two detection channels are measured. On the EnVision XCite, this is done subsequently while on the EnVision HTS, a simultaneous readout is performed, which requires a different mirror module.

It is possible to use single mirror modules on the EnVision HTS, but not vice versa!

Assay Type	Emission filter	2nd emission filter	Mirror Module XCite	Mirror Module HTS
<b>BRET</b>	2100-5870 YFP 530/35 filter	2100-5040 FITC FP 480/30 filter	2100-4040 Luminescence -/- single mirror	2100-4110 Luminescence -/bs50 dual mirror
<b>BRET2</b>	2100-5300 BRET2 515/30 filter	2100-5290 BRET2 410/80 filter	2100-4040 Luminescence -/- single mirror	2100-4150 BRET2 -/D475 dual mirror
<b>Chroma-Glo</b>	2100-5970 BRET Deep Red 647/75	2100-5960 ChromaGlo Green 510/60	2100-4040 Luminescence -/- single mirror	2100-4380 ChromaGlo/NanoBRET
<b>FireFly Luminescence</b>	2100-5180 Luminescence <700 emission filter	-	2100-4040 Luminescence -/- single mirror	2100-4040 Luminescence -/- single mirror
<b>NanoBRET</b>	2100-5970 BRET Deep Red 647/75	2100-5950 NanoBRET Blue 460/80	2100-4040 Luminescence -/- single mirror	2100-4380 ChromaGlo/NanoBRET
<b>Renilla Luciferase</b>	2100-5180 Luminescence <700 emission filter	-	2100-4040 Luminescence -/- single mirror	2100-4040 Luminescence -/- single mirror
<b>Twinlite</b>	2100-5180 Luminescence <700 emission filter	-	2100-4040 Luminescence -/- single mirror	2100-4040 Luminescence -/- single mirror

## Choosing the best filter combination

### Step 1: Know your fluorophore

Very often, for fluorophores only the peak excitation and emission wavelengths are noted, but in truth, the spectra are much broader, like shown in Figure 1. The shape sketched here is only a typical representation and in reality the spectra of dyes could be more complex. Try to find more information about the fluorophore than only peak wavelengths; a complete excitation/emission spectrum is the best to start with.

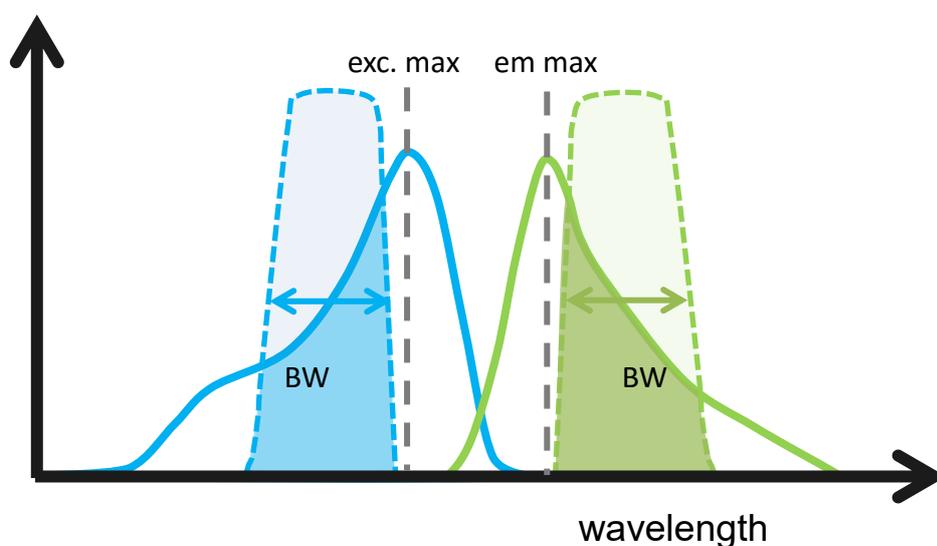


Figure 1: Schematic representation of excitation and emission spectra of a fluorophore (blue and green solid lines, respectively), together with the transmission spectra of excitation and emission filters (dashed blue and green lines, respectively). BW denotes the filter bandwidth

### Step 2: Know your filters

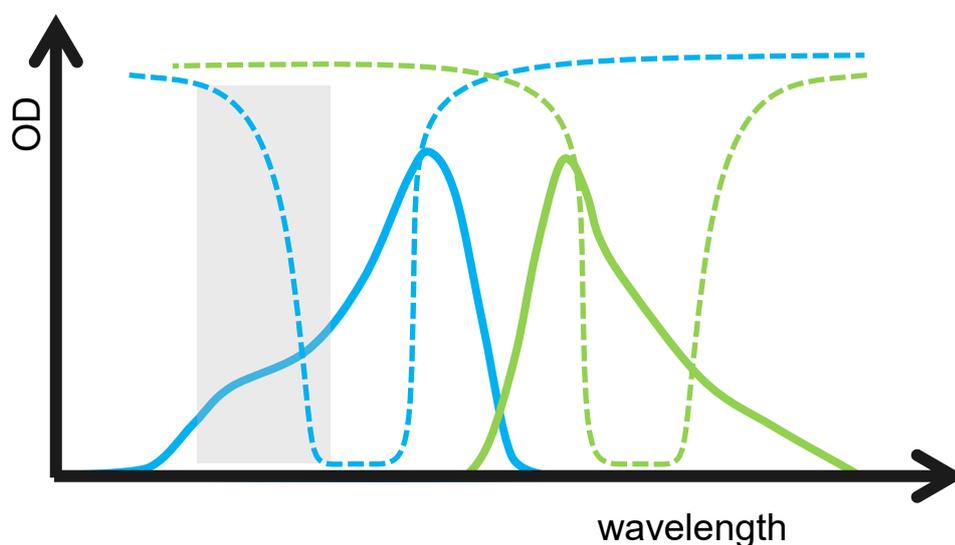
As is the case with fluorophores, optical filters are not only fully characterized by their central wavelengths, either. At least the bandwidth (BW) of filters needs to be taken into account. This is the width of the filter along the wavelength axis, usually measured at half the filter's maximum transmission value (see Figure 1), the so called full-width-at-half-maximum (FWHM). It describes the wavelength range the filter can transmit. The larger it is the more light can pass. The drawback is that a broader bandwidth also allows light from other wavelengths to pass through.

### Step 3: Combine excitation and emission filters with the fluorophore

A good filter choice for a given fluorophore is where the area of the fluorophore's spectrum and the filter's transmission range have the largest overlap. This is depicted as the blue-coloured area in Figure 1. It gets larger if a range with a higher spectrum curve is covered, but it also increases when the filter bandwidth is increased. So the obvious approach would be to choose a filter which would cover the whole spectrum of a fluorophore – but why is this not done usually? The reason here is that a filter needs to serve another purpose than only transmitting light: it needs to block unwanted light. An excitation filter needs a large transmission capability where at the fluorophore's excitation range, so that excitation light from the light source can reach the sample. However, excitation light outside this range should not be transmitted, since it could be reflected

on e.g. the sample surface and reach the detector. At the same time, an emission filter needs to have a large transmission value where the fluorophore has its emission range, but it has to block the actual excitation light.

This is visualized in Figure 2, where the filter blocking is sketched together with the fluorophore's spectra. The transition between blocking range and transmission range does not have a rectangular shape (see e.g. shaded area in Figure 2).



**Figure 2: Schematic representation of excitation and emission spectra of a fluorophore (solid lines) together with the Optical Density of the filters from Figure 1 (dashed lines).**

The excitation filter has a low optical density and thus a high transmission at the excitation range of the fluorophore, but the blocking increases to higher wavelengths around the emission range. The emission filter shows a high OD value and therefore a good blocking at the excitation range and a low blocking (=high transmission) at the emission range. Note that at the point where the OD curves of the filters intersect, both filters need to have high OD values for sufficient blocking. For Fluorescence filters, an OD of 5 or higher is preferred at this point. The steeper the slopes of the filter OD curves are, the closer two filters can be moved with respect to each other.

#### **Step 4: Work with the list of available filters**

Many filters are already available as catalogue filters. Without the exact knowledge of the transmission curves of filters, finding a matching filter pair can be challenging. However, a good rule of thumb is that the excitation filter central wavelength + excitation filter bandwidth must be smaller than the emission filter central wavelength – emission filter bandwidth. For many fluorescence applications filter bandwidth of 15-30nm are appropriate. Much broader filters are only in special cases superior as they also allow or a higher level of potential background signal e.g. caused by autofluorescence. A general observation is that broader filters give a better signal-to-noise ratio and in turn a better sensitivity compared to narrower filters, but might show a lower signal-to-background ratio.