VICTOR® Nivo™ multimode plate reader

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How to use this list

Search for the wavelength (“Numerical Wavelength List”), the fluorophore (“Alphabetical Dye List”) or assay label you want to use in one of the lists and find the suggested filter and dichroic mirror combination.

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 (HH35000902)” 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 down to 230nm are reflecting excitation light to the sample, wavelength ranges above up to 850nm are transmitting emission light from the sample to the detector. The use of a dedicated dichroic mirror in Fluorescence and Fluorescence polarization assays is optional, but gives better results than the Standard 50/50 beam splitter.

Please note that there is no need for filter central wavelength and the peak wavelengths usually given for fluorophores to be identical. If you need more information on choosing the correct filter combination for your fluorophore, please have a look at the end of this document, page 7.

Numerical wavelength list including preferred technologies

Name	Part number	CWLi / nm	BWii / nm	Pref. tech.	Application examples
EMPTY	HH35000900				Empty Filter Holder for custom optics
260/10nm	HH35000910	260	10	ABS	Nucleotides (intrinsic absorbance)
280/10nm	HH35000911	280	10	ABS, FI	Protein (intrinsic absorbance), Trp
320/75nm	HH35000947	320	75	TRF	General TRF Excitation filter
340/20nm	HH35000912	340	20	FI, FP	Fura-2
355/40nm	HH35000913	355	40	FI, FP	Alexa 350, AMC, BFP, DAPI, Hoechst 33342, Trp
380/20nm	HH35000914	380	20	FI, FP	Fura-2
390/20nm	HH35000915	390	20	FI	Fluorescamine
405/10nm	HH35000901iii	405	10	ABS	ELISA (PNPP, ABTS)
410/80nm	HH35000916	410	80	LUM	BRET2
420/10nm	HH35000917	420	10	ABS	ELISA (ABTS)
435/20nm	HH35000918	435	20	FI, FP	CFP, Citrine, Cerulean
450/10nm	HH35000919	450	10	ABS	ELISA (TMB)
460/30nm	HH35000921	460	30	FI, FP	Alexa 350, AMC, BFP, DAPI, Hoechst 33342, BRET, Fluorescamine
460/80nm	HH35000920	460	80	LUM	NanoBRET
480/30nm	HH35000902iii	480	30	FI, FP	FITC, Alexa 488, Calcein, DyLight 488, Fluo-4, GFP, PicoGreen, SybrGreen
492/10nm	HH35000948	492	10	ABS	ELISA (OPD), LDH Activity Assay (Formazan based)
495/20nm	HH35000922	495	20	FI, FP, TRF	Venus, YFP, HTRF Assays, LanthaScreen
510/30nm	HH35000923	510	30	FI, FP	Fura-2
510/60nm	HH35000924	510	60	LUM	Chroma-Glo
515/30nm	HH35000925	515	30	LUM	BRET
520/25nm	HH35000949	520	25	TRF	HTRF Assays, LanthaScreen
530/30nm	HH35000903iii	530	30	FI, FP	FITC, Alexa 488, Calcein, DyLight 488, Fluo-4, GFP, PicoGreen, SybrGreen, 5-TAMRA, Alamar Blue, Alexa 555, Amplex red, Bodipy-TMR, Cy3, dsRed, mOrange, RFP, Resorufin, TRITC
540/10nm	HH35000930	540	10	FI	Alamar Blue
540/30nm	HH35000926	540	30	FI, FP	Venus, YFP
546/11nm	HH35000927	546	11	TRF, ALPHA	DELFIA (Tb Assays), AlphaPlex
560/10nm	HH35000928	560	10	ABS	BCA Protein
570/10nm	HH35000929	570	10	ABS	MTT
575/110nm	HH35000950	575	110	ALPHA	AlphaLISA, AlphaScreen, Alpha Surefire
580/10nm	HH35000931	580	10	ABS	Methyl-resorufin, starch-iodine, Copper(DiBr-PAESA)
580/20nm	HH35000932	580	20	FI	5-TAMRA, Alamar Blue, mOrange
595/10nm	HH35000933	595	10	ABS	Bradford Assay

Numerical wavelength list including preferred technologies (continued)

Name	Part number	CWLi / nm	BWii / nm	Pref. tech.	Application examples
600/10nm	HH35000934	600	10	ABS	Bacteria, cells (intrinsic)
615/8nm	HH35000935	615	8	TRF, ALPHA	DELFIA, LANCE, Europium Assays, LanthaScreen, AlphaPlex
620/10nm	HH35000936	620	10	TRF, ABS	HTRF Assays, ELISA (reference)
625/30nm	HH35000937	625	30	FI, FP	Alexa 568, Alexa 594, mCherry, Texas Red
640/30nm	HH35000938	640	30	FI, FP	Alexa 647, APC, Cy5
644/10nm	HH35000939	644	10	TRF, ALPHA	DELFIA (Samarium Assays), AlphaPlex
645/75nm	HH35000940	645	75	LUM	NanoBRET
650/10nm	HH35000941	650	10	ABS	ELISA (ABTS), Lowry Protein Assay
660/10nm	HH35000942	660	10	ABS	Pierce Protein Assay, Chlorophyll
665/8nm	HH35000943	665	8	TRF	LANCE , HTRF Assays, LanthaScreen
685/30nm	HH35000944	685	30	FI, FP	Alexa 647, APC, Cy5
690/10nm	HH35000945	690	10	ABS	MTT (reference), Phosphate
700nm sp	HH35000904iii	700	short pass	LUM	Generic Luminescence filter
740/40nm	HH35000951	740	40	FI	Alexa 750, Alexa 790, Cy7, IRDye 800, DyLight 800, Indocyanine green (ICG)
750/10nm	HH35000946	750	10	ABS	Lowry Protein Assay, Pierce Protein Assay (reference)
780nm lp	HH35000952	780	long pass	FI	Alexa 750, Alexa 790, Cy7, IRDye 800, DyLight 800, Indocyanine green (ICG)

Dichroic mirrors:

Name	Part number	Cut-on wavelength /nm	Example applications
BS50/50	HH35000970iii	50/50	all FI, FP, and TRF
D400	HH35000971	400	Alexa 350, AMC, BFP, DAPI, Hoechst 33342, DELFIA, LANCE, HTRF Assays, LanthaScreen
D455	HH35000972	455	CFP, Cerulean
D500	HH35000973	500	FITC, Alexa 488, Calcein, Cy2, DyLight 488, Fluo- 4, GFP, PicoGreen, SybrGreen, Venus, YFP
D565	HH35000974	565	5-TAMRA, Amplex Red, Alamar Blue, Alexa 555, Bodipy-TMR, Cy3, dsRed, mOrange, RFP, Resorufin, TRITC
D590	HH35000975	590	Alexa 568, Alexa 594, mCherry, Texas Red
D660	HH35000976	660	Alexa 647, APC, Cy5
D660A	HH35000977	660	Alpha (not applicable for other technologies!), e.g. AlphaLISA, AlphaScreen, Alpha Surefire, AlphaPlex
D770	HH35000978	770	Alexa 750, Alexa 790, Cy7, IRDye 800, DyLight 800, Indocyanine green (ICG)

Alphabetical dye list for fluorescence or fluorescence polarization

Fluorophore	Excitation filter	Emission filter	Dichroic mirror
5-TAMRA	530/30nm (HH35000903)	580/20nm (HH35000932)	D565 (HH35000974)
Alamar Blue	530/30nm (HH35000903) or 540/10nm (HH35000030)*	580/20nm (HH35000932)	D565 (HH35000974)
Alexa 350	355/40nm (HH35000913)	460/30nm (HH35000921)	D400 (HH35000971)
Alexa 488	480/30nm (HH35000902)	530/30nm (HH35000903)	D500 (HH35000973)
Alexa 555	530/30nm (HH35000903)	580/20nm (HH35000932)	D565 (HH35000974)
Alexa 568	580/20nm (HH35000932)	625/30nm (HH35000937)	D590 (HH35000975)
Alexa 594	580/20nm (HH35000932)	625/30nm (HH35000937)	D590 (HH35000975)
Alexa 647	640/30nm (HH35000938)	685/30nm (HH35000944)	D660 (HH35000976)
Alexa 750	740/40nm (HH35000951)	780nm lp (HH35000952)	D770 (HH35000978)
Alexa 790	740/40nm (HH35000951)	780nm lp (HH35000952)	D770 (HH35000978)
AMC (7-Amino-4- Methylcoumarin)	355/40nm (HH35000913)	460/30nm (HH35000921)	D400 (HH35000971)
Amplex Red	530/30nm (HH35000903)	580/20nm (HH35000932)	D565 (HH35000974)
APC (Allophycocyanin)	640/30nm (HH35000938)	685/30nm (HH35000944)	D660 (HH35000976)
Blue Fluorescent Protein (BFP)	355/40nm (HH35000913)	460/30nm (HH35000921)	D400 (HH35000971)
Bodipy-TMR	530/30nm (HH35000903)	580/20nm (HH35000932)	D565 (HH35000974)
Calcein	480/30nm (HH35000902)	530/30nm (HH35000903)	D500 (HH35000973)
Cerulean	435/20nm (HH35000918)	480/30nm (HH35000902)	D455 (HH35000972)
Cyan Fluorescent Protein (CFP)	435/20nm (HH35000918)	480/30nm (HH35000902)	D455 (HH35000972)
Citrine	435/20nm (HH35000918)	480/30nm (HH35000902)	D455 (HH35000972)
Cy3	530/30nm (HH35000903)	580/20nm (HH35000932)	D565 (HH35000974)
Cy5	640/30nm (HH35000938)	685/30nm (HH35000944)	D660 (HH35000976))
Cy7	740/40nm (HH35000951)	780nm lp (HH35000952)	D770 (HH35000978)
DAPI	355/40nm (HH35000913)	460/30nm (HH35000921)	D400 (HH35000971)
dsRED	530/30nm (HH35000903)	580/20nm (HH35000932)	D565 (HH35000974)
DyLight 488	480/30nm (HH35000902)	530/30nm (HH35000903)	D500 (HH35000973)
DyLight 800	740/40nm (HH35000951)	780nm lp (HH35000952)	D770 (HH35000978)
FITC	480/30nm (HH35000902)	530/30nm (HH35000903)	D500 (HH35000973)
Fluo-4	480/30nm (HH35000902)	530/30nm (HH35000903)	D500 (HH35000973)
Fluorescamine	390/20nm (HH35000915)	460/30nm (HH35000921)	D400 (HH35000971)
Fura-2	340/20nm (HH35000912) 380/20nm (HH35000914)	510/30nm (HH35000923)	D400 (HH35000971)
(e)GFP: (enhanced) Green Fluorescent Protein	480/30nm (HH35000902)	530/30nm (HH35000903)	D500 (HH35000973)
Hoechst 33342	355/40nm (HH35000913)	460/30nm (HH35000921)	D400 (HH35000971)
Indocyanine green (ICG)	740/40nm (HH35000951)	780nm lp (HH35000952)	D770 (HH35000978)

Alphabetical dye list for fluorescence or fluorescence polarization (continued)

Fluorophore	Excitation filter	Emission filter	Dichroic mirror
IRDye 800	740/40nm (HH35000951)	780nm lp (HH35000952)	D770 (HH35000978)
mCherry	580/20nm (HH35000932)	625/30nm (HH35000937)	D590 (HH35000975)
mOrange	530/30nm (HH35000903)	580/20nm (HH35000932)	D565 (HH35000974)
PicoGreen	480/30nm (HH35000902)	530/30nm (HH35000903)	D500 (HH35000973)
Red Fluorescent Protein (RFP)	530/30nm (HH35000903)	580/20nm (HH35000932)	D565 (HH35000974)
Resorufin	530/30nm (HH35000903)	580/20nm (HH35000932)	D565 (HH35000974)
SybrGreen	480/30nm (HH35000902)	530/30nm (HH35000903)	D500 (HH35000973)
Texas Red	580/20nm (HH35000932)	625/30nm (HH35000937)	D590 (HH35000975)
TRITC	530/30nm (HH35000903)	580/20nm (HH35000932)	D565 (HH35000974)
Tryptophan (Trp)	280/10nm (HH35000911)	355/40nm (HH35000913)	BS50/50 (HH35000970)
Venus	495/20nm (HH35000922)	540/30nm (HH35000926)	D500 (HH35000973)
Yellow Fluorescent Protein (YFP)	495/20nm (HH35000922)	540/30nm (HH35000926)	D500 (HH35000973)

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

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 find a spectral analogue which is listed here.

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

Alphabetical assay list for absorbance

Chromophore	Filter (or spectrometer setting)
Bacteria (intrinsic)	600/10nm (HH35000934)
BCA	560/10nm (HH35000928)
Bradford	595/10nm (HH35000933)
Cells (intrinsic)	600/10nm (HH35000934)
ELISA: ABTS	405/10nm (HH35000901)
ELISA: OPD	492/10nm (HH35000948)
ELISA: PNPP	405/10nm (HH35000901)
ELISA: TMB	450/10nm (HH35000919)
LDH Activity Assay (Formazan based)	492/10nm (HH35000948)
Nucleic Acids (unlabeled)	260/10nm (HH35000910)
Pierce Protein Assay	660/10nm (HH35000942)
Protein (unlabeled)	280/10nm (HH35000911)
Lowry Protein Assay	750/10nm (HH35000946) or 650/10nm (HH35000941)
Methyl-resorufin	580/10nm (HH35000931)
MTT Assay	570/10nm (HH35000929)

Please refer to your assay description for suggested filters. Usually, the center wavelengths are given there. Do not use broad FI filters for Absorbance. This will lead to unreliable results and instrument error notifications.

Dye list for Time-Resolved Fluorescence (TRF)

Assay Type	Excitation filter	Emission filter	2nd emission filter	Dichroic mirror
DELFIA	320/75nm (HH35000947)	615/8nm (HH35000935)	–	D400 (HH35000971)
LANCE	320/75nm (HH35000947)	615/8nm (HH35000935)	665/8nm (HH35000943)	D400 (HH35000971)
HTRF (Eu or Tb/Red)	320/75nm (HH35000947)	620/10nm (HH35000936)	665/8nm (HH35000943)	D400 (HH35000971)
HTRF (Tb/Green)	320/75nm (HH35000947)	*495/20nm (HH35000922)	520/25nm (HH35000949)	D400 (HH35000971)
LanthaScreen (Eu or Tb/FITC or GFP)	320/75nm (HH35000947)	495/20nm (HH35000922)	520/25nm (HH35000949)	D400 (HH35000971)
LanthaScreen (Eu/ Alexa647)	320/75nm (HH35000947)	615/8nm (HH35000935)	665/8nm (HH35000943)	D400 (HH35000971)

**620/10nm (HH35000936) can also be used.*

Alphabetical dye list luminescence

Assay Type	Emission filter	2nd emission filter
BRET	460/30nm (HH35000921)	515/30nm (HH35000925)
BRET2	410/80nm (HH35000916)	515/30nm (HH35000925)
Chroma-Glo	510/60nm (HH35000924)	645/75nm (HH35000940)
FireFly Luminescence	700nm sp	–
NanoBRET	460/80nm (HH35000920)	645/75nm (HH35000940)
Renilla Luciferase	700nm sp (HH35000904)	–
Twinlite	700nm sp (HH35000904)	–

Custom Optics Service

Please contact your local Revvity representative for a customized solution for your assay.

i CWL = central wavelength of bandpass filter
ii BW = bandwidth of filter
iii Shipped with every VICTOR Nivo reader

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.

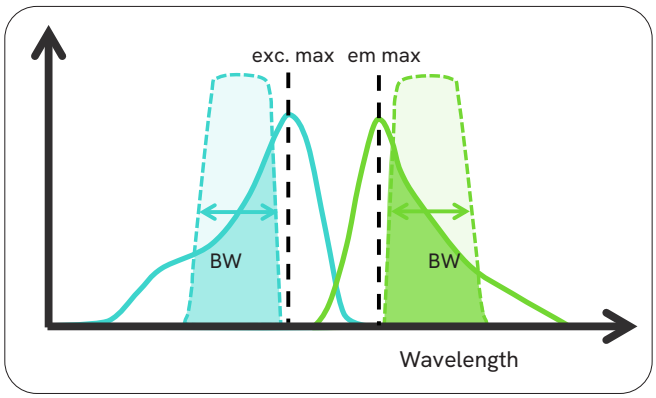

Figure 1: Schematic representation of excitation and emission spectra of a fluorophore (blue and green solid lines, respectively), together with the transmission spectra of excitation and emission filters (dashed blue and green lines, respectively). BW denotes the filter bandwidth.

Try to find more information about the fluorophore than only peak wavelengths, a complete excitation/emission spectrum is the best to start with.

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-colored 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 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).

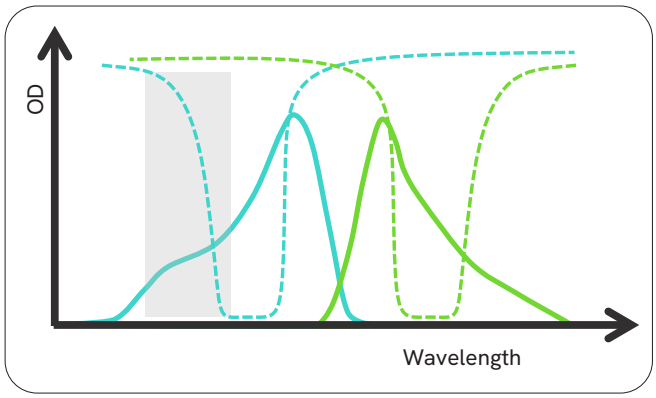

Figure 2: Schematic representation of excitation and emission spectra of a fluorophore (solid lines) together with the Optical Density of the filters from Figure 1 (dashed lines).

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 for 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.

Revvity, Inc.
940 Winter Street
Waltham, MA 02451 USA

(800) 762-4000
www.revvity.com