Fluorochromes Description

7AAD:

Excited by 488nm argon laser. 7AAD binds selectively to GC regions of DNA. 7AAD is used as a viability dye to discriminate between viable and nonviable cells.

Alexa Fluor 350:

Excited by 355nm UV laser. Alexa Fluor 350 is a blue-fluorescent dye that produces conjugates that are typically greater than 50% more fluorescent than conjugates prepared with AMCA. Alexa Fluor 350 has slightly shorter-wavelength emission maxima than AMCA conjugates, the fluorescence of Alexa Fluor 350 conjugates is better separated from that of commonly used green fluorophores.

Alexa Fluor 405:

Excited by 405nm violet laser. Alexa Fluor 405 is an extremely dim dye, for that reason it should be only used for the brightest antigens. It is a derivated of the Cascade Blue dye. Alexa Fluor 405 conjugates show minimal spectral overlap with green fluorophores and is potentially brighter than UV light-excitable blue fluorophores, whose signal is often obscured by autofluorescence.

Alexa Fluor 430:

Excited by 405nm violet laser. Alexa Fluor 430 is one of the few dyes that absorb between 400nm and 450nm and emit an appreciable fluorescence beyond 500nm. It exhibits a dim yellow green fluorescence. Although Alexa Fluor 430 is a dim dye it remains an interesting choice for the violet laser for its specific spectral properties and also because of his high photostability and pH-insensitive fluorescence.

Alexa Fluor 488:

Excited by 488nm argon laser. Alexa Fluor 488 conjugates are highly photostable and remain fluorescent over a broad pH range. It is interchangeable with FITC since the fluorescence spectra is almost identical to those of fluorescein with only minor changes in compensation. Alexa Fluor 488 is a very good choice for flow cytometry.

Alexa Fluor 500:

Excited by 488nm argon laser. Alexa Fluor 500 is a green-fluorescent fluorochrome that is specifically designed to de detected simultaneously with other green fluorophores, despite its spectral overlap. Alexa Fluor 500 is also very photostable and pH-insensitive.

Alexa Fluor 514:

Excited by 488nm argon laser. Like Alexa Fluor 500, Alexa Fluor 514 is a green-fluorescent fluorochrome that is specifically designed to de detected simultaneously with other green fluorophores, despite its spectral overlap. Alexa Fluor 514 is also very photostable and pH-insensitive. Alexa Fluor 500 and 514 can be separated optically using specific spectral imaging instruments.

Alexa Fluor 532:

Excited by a 488nm argon laser. Alexa Fluor 532 is a fluorophore that exhibits strong visible fluorescent between those of green fluorophores and orange fluorophores. It has a strong absorption of photons and is more photostable than other spectrally similar dyes. The high photostability is due to a broad range pH-insensitivity.

Alexa Fluor 555:

Excited by a 488nm argon laser or a 546nm mercury-arc lamp. Alexa Fluor 555 is a dim fluorophore. Altough it's a weak fluorophore, despite his high absorption property, it remains a good choice instead of Cy3 since Alexa Fluor 555 is far more photostable.

Alexa Fluor 546:

Excited by 561nm yellow green laser. Alexa Fluor 546 is a great choice for applications that require fluorescent probes that emit in the orange region of the spectrum .These intensely fluorescent conjugates outperform the conjugates of Cy3. They also show a strong photostability and a pH-insensitive fluorescence over a broad PH range.

Alexa Fluor 568:

Excited by 561nm yellow-green laser. Alexa Fluor 568 is a red-orange fluorescent dye that is producing a bright fluorescence that contrast well with the green fluorescence of FITC or Alexa Fluor 488. Alexa Fluor 568 is a very photostable dye with pH-insensitive fluorescence over a broad pH range.

Alexa Fluor 594:

Excited by a 561 nm yellow-green laser. Alexa Fluor 594 emits in the red region of the spectrum. His main advantage is to be much photostable than other red fluorophores particularly Texas Red conjugates.

Alexa Fluor 610:

Excited by 561nm yellow-green laser. Alexa Fluor 610 is a bright and photostable fluorochrome that emits a red fluorescence visualized with the same optics used for Texas Red and Alexa Fluor 594 dyes. It can be easily differentiated from green fluorophores, making it an ideal candidate for multicolor labeling.

Alexa Fluor 633:

Excited by 633 He-Ne laser. Alexa Fluor 633 is a far-red fluorescent dye. This dye is bright and photostable. Although it's not as bright as Alexa Fluor 647, it remains a good fluorochrome.

Alexa Fluor 647:

Excited by 633nm He-Ne laser. Alexa Fluor 647 conjugates virtually match those of the Cy5 dye, resulting in an optical match to optical filters designed for that dye. It is an excellent alternative to APC because of his extremely bright fluorescence and pH insensitivity. Furthermore, Alexa Fluor 647 exhibits uncommon photostability.

Alexa Fluor 660:

Excited by 633nm He-Ne laser. Alexa Fluor 660 is better excited by the 647nm line of the krypton laser but it can be used with a 633nm He-Ne laser. It exhibits a bright fluorescence in the far red region of the spectrum. Due to its photostability and its pH-insensitivity, Alexa Fluor 660 is a very good choice to combine with APC for He-NE laser.

Alexa Fluor 680:

Excited by 633nm He-Ne laser. Alexa Fluor 680 is spectrally similar to the Cy5.5 dye. Fluorescence emission of the Alexa Fluor 680 dye is well separated from that of other commonly used red fluorophores, such as the Texas Red, PE, Alexa Fluor 594 and Alexa Fluor 647 dyes. Among the Alexa Fluor family, Alexa Fluor 680 is one of the brightest. It also benefits from the high photostability and pH-insensitivity of the Alexa Fluor family.

Alexa Fluor 700:

Excited by 633nm He-Ne laser or red diode laser. Alexa Fluor 700 is a far-red dye that can be used in conjunction of APC, Alexa Fluor 647, APC-Cy7 or APC-Alexa Fluor 750 reagents for the red region of the spectrum. Although not optimally excited by the He-Ne laser, it produces a bright signal that can be used as a third dye off this laser. Like all Alexas it is extremely photostable and has no compensation against APC.

AmCyan:

Excited by 405nm violet laser. AmCyan is a protein derivated from *Anemonia majano*. Although AmCyan is a very dim dye, its association with Pacific Blue for the violet laser allows to have a good combination of dyes for the violet laser.

APC (Allophycocyanin):

Excited by 633nm He-Ne laser. APC is a photosynthetic pigment found in bluegreen algae. can be used interchangeably with Cy5 or Alexa 647. APC exhibits a very bright fluorescence in the far red region of the spectrum.

APC-Alexa Fluor 750:

Excited by 633nm He-Ne laser. APC-Alexa Fluor 750 is more photostable than APC-Cy7. It exhibits a bright fluorescence in the far-red region of the spectral and gives less compensation against APC than APC-Cy7.

APC-Cy5.5:

Excited by 633nm He-Ne laser. APC-Cy5.5 is a tandem conjugate that uses the resonance energy transfer from APC to Cy5.5. It exhibits a strong fluorescence in the far red region of the spectrum. Like all tandems conjugates, APC-Cy5.5 is to be used with precaution as it is subject to uncoupling and photobleaching.

APC-Cy7:

Excited by 633nm He-Ne laser. APC-Cy7. APC-Cy7 is also a tandem conjugate associating APC to Cy7. It is the tandem conjugate that emits the longest-wavelength fluorescence. Although it's a good choice for red lasers as it's one of the rare fluorochrome to emits in the far red region of the spectrum, it remains a dim tandem dye that could be chosen for highly expressed protein/antigens of interest. Special precautions must be taken when using APC-Cy7 to avoid long-term exposure to visible light. It is advised to fix the cells stained with APC-Cy7.

APC-H7:

Excited by 633nm He-Ne laser. APC-H7 is a APC-cyanine tandem dye that is analog to APC-Cy7 with similar spectral properties. It has a greater stability in light and paraformaldehyde fixatives and generates less spillover in the APC channel. Nevertheless, APC-H7 conjugates are typically 25% less bright than equivalent APC-Cy7 conjugates.

Cascade Blue:

Excited by 355nm UV laser. Cascade Blue is a UV-excitable dye that can be used for immunofluorescence labeling. When used with the 351/361 nm excitation lines of an Argon laser, it is not very bright; usually only extremely high density antigens can be well-resolved by Cascade Blue. However, when used with the 405nm excitation line of a Krypton laser, it becomes a useful dye with a brightness approaching that of fluorescein.

Cascade Yellow:

Excited by 405nm argon laser. Cascade yellow is a fluorochrome that exhibits an excitation maximum that falls between those of the UV light–excited dyes and the fluoresceins. This dye has a large stoke shift that permits detection at a wavelength well beyond that of most sample autofluorescence, and allows multiple fluorophores to be excited at the same wavelengths and detected at different wavelengths.

Cy3:

Excited by 488nm argon laser. Cy3 is a fluorescent dye of the cyanine dye family. Cy3 dyes are yellow-orange. Specific filter wavelengths (550-600 nm and 655-695 nm) have to be used to avoid background contamination when using Cy3 and Cy5 together to optically separate the two fluorescent cyanines. Cy3 is used interchangeably with PE, though PE remains a better choice.

Cy5:

Excited by 633nm He-Ne laser. Cy5 can be used interchangeably with APC or Alexa Fluor 647. It is typically the dye used for intracellular labeling. Cy5 emits a very bright fluorescence in the infrared region of the spectrum. Although Cy5 is a good choice it remains far less photostable than the Alexa Fluor Family emitting in the same region. It shows also a greater fluorescence quenching upon conjugation to proteins.

Cy7:

Excited by 633nm He-Ne laser. Cy7 is a cyanine dye commonly used as fluorescent label for proteins, nucleic acids and small molecules. Cyanine Dye 7 (Cy7) exhibits a strong fluorescence in the far-red region of the spectrum. It can be used as an alternative to Alexa Fluor 750.

DAPI:

Excited by 355nm UV laser. DAPI is a fluorescent stain that binds strongly to DNA. Since DAPI will pass through an intact cell membrane, it may be used to stain both live and fixed cells. DAPI will also bind to RNA, though it is not as strongly fluorescent. There is a fluorescence overlap between DAPI and green-fluorescent molecules like FITC and GFP, or red-fluorescent fluorescent proteins like Texas Red.

DsRed:

Excited by 561nm yellow-green laser. DsRed is a mutant of the red fluorescent protein from *Discosoma sp.* reef coral. The DsRed protein is extremely stable and exhibits a very bright fluorescence in the red region of the spectrum.

ECFP (Enhanced Cyan Fluorescent Protein):

Excited by 405nm violet laser. ECFP is a fluorescent protein emitting in the bluish-green cyan spectral region. A single mutation of the CFP protein resulted in the production of an enhanced version ECFP with greater brightness and photostability. Nevertheless ECFP is not as bright as EGFP and EYFP.

EGFP (Enhanced Green Fluorescent Protein):

Excited by 488nm argon laser. EGFP is obtained from a single mutation froe the original green fluorescent protein isolated from *Aequorea victoria*. EGFP can be detected using commonly available filter sets designed for FITC. These features have rendered EGFP one of the most popular probe. The only drawbacks to the use of EGFP as a fusion tag are a slight sensitivity to pH and a weak tendency to dimerize.

EYFP (Enhanced Yellow Fluorescent Protein):

Excited by 488nm argon laser. EYFP is one of the brightest and most widely utilized fluorescent proteins. EYFP was constructed from the original yellow variant by mutation. The high brightness level and fluorescence emission spectrum wavelength profile of EYFP combine to make this protein an excellent choice for fused proteins. However, EYFP is very sensitive to acidic pH and lose approximately 50 percent of their fluorescence at pH 6.5. EYFP is also sensitive to chloride and is subject to photobleach.

FITC (Fluorescein):

Excited by 488nm argon laser. FITC is the most widely used form for conjugation to antibodies and proteins, but other derivatives are available. FITC gives a decent fluorescent signal in the green region of the spectrum. Though, precaution must be taken when using FITC as it is sensitive to pH changes and photobleaching.

Marina Blue:

Excited by 355nm UV laser. Marina Blue is a dim fluorophore emitting in the blue region of the spectrum. This fluorophore is not particularly efficient for violet excitation. Marina Blue dyes yield conjugates that are strongly fluorescent, even at neutral pH.

mBanana:

Excited by 561nm yellow-green laser. mBanana is the dimmest Fruit Fluorescent Protein. It emits a very dim fluorescence in the yellow region of the spectrum. Its maximum excitation peak being found in between two lines of excitation of the blue argon laser and the yellow-green laser, mBanana is a poor choice considering its weak spectral properties.

mCherry:

Excited by a 561nm yellow green laser. mCherry is a dim fluorescent protein used as a tag protein for many applications. mCherry is particularly used for in vivo imaging because far red proteins are preferred to avoid the natural green autofluorescence produced by animal cells. It emits a dim fluorescence in the red region of the spectrum. It is detected with the same filter sets as PE-Texas Red and Alexa Fluor 568.

mHoneyDew:

Excited by 488nm argon laser. mHoneyDew fits perfectly for the 488nm laser line. It emits a very dim green-yellowish fluorescence. A very poor choice unless it's chosen for its specific spectral properties (region of emission).

mOrange:

Excited by 561nm yellow-green laser. mOrange is the brightest proteins from the Fruit Fluorescent Protein family. It exhibits a strong fluorescence in the orange region of the spectrum. This protein can be used instead of RFP or Ds Red proteins.

mPlum:

Excited by 561nm yellow-green laser. mPlum is an interesting choice in the RFP variants since it emits a fluorescence in the far-red region of the spectrum and has interesting spectral properties. Nevertheless, a very low quantum yield renders this protein a poor choice for a Tag protein as the intensity of the fluorescence is very weak. Only mHoneyDew has a weaker fluorescence.

mRaspberry:

Excited by 561nm yellow-green laser. mRaspberry belongs to the RFP variants family. It exhibits a dim fluorescence in the far-red region of the spectrum. Although, mRaspberry has a great extinction coefficient, its quantum yield is too weak to make mRaspberry a good choice for a Tag protein. mStrawberry or mOrange are two better choice for almost similar spectral properties.

mStrawberry:

Excited by 561nm yellow-green laser. mStrawberry is a fluorescent protein that belongs to the Fruit Fluorescent Protein. It exhibits a bright fluorescence in the red region of the spectrum. mStrawberry is especially recommended to use in the detection of Protein-Protein Interactions (FRET).

mTangerine:

Excited by 561nm yellow-green laser. mTangerine is issue from the Fruit Fluorescent Proteins family or RFP variants. Those proteins are obtained by mutations of the RFP protein. mTangerine has a decent spectral properties but remains a weak choice as it exhibits a dim Orange-redish fluorescence.

Oregon Green 488:

Excited by 488nm argon laser. Oregon Green 488 is a fluorophore that mimics spectral properties of fluorescein without having a high rate of photobleaching. Furthermore Oregon Green 488 is much more photostable than fluorescein and have less or no pH sensitivity. When compared with fluorescein, this dye exhibits the same or slightly longer-wavelength spectra and comparably high fluorescence quantum yield. It’s a really good choice instead of FITC for the argon laser.

Pacific Blue:

Excited by 405nm violet laser. Pacific Blue is strongly fluorescent even at neutral pH. It can be used interchangeably with Alexa Fluor 405 which is typically a dimmer fluorochrome. Pacific Blue has minimal spectral overlap with the green fluorochromes. Nevertheless, it remains a dim fluorochrome that needs to be used with the highly expressed protein/antigen of interest.

Pacific Orange:

Excited by 405nm violet laser. Pacific Orange is a dye fully compatible with Pacific Blue and can be used instead of AmCyan. Pacific Orange emits a dim fluorescence in the region between the green and the orange region of the spectrum.

PE (R-Phycoerythrin):

Excited by 488nm argon laser. PE is an accessory photosynthetic pigment found in the red algae. PE is subject to photobleaching but emits a very intense fluorescence in the red region of the spectrum. It is an ideal choice for the detection of low density antigens.

PE-Alexa Fluor 610:

Excited by 488nm argon laser or 561nm yellow-green laser. PE-Alexa Fluor 610 is a tandem dye using the resonance energy transfer from PE to Alexa Fluor 610. It is a bright and photostable fluorochrome that emits a red fluorescence visualized with the same optics used for Texas Red and Alexa Fluor 594 dyes.

PE-Cy5:

Excited by 488nm argon or 561nm yellow-green laser. PE-Cy5 is a tandem dye, using the resonance energy transfer from PE to the cyanine dye Cy5. The efficiency of the light transfer between the two fluorochromes allows less than 5% of the absorbed light to be lost as fluorescence by PE. Because of his broad absorption range, PE-Cy5 is not recommended for use with dual-laser flow cytometers where excitation by both lasers is possible. PE-Cy5 is also subject to photobleaching and uncoupling.

PE-Cy5.5:

Excited by 488nm argon laser or 561nm yellow green laser. PE-Cy5.5 is a tandem dye using the resonance energy transfer from PE to the cyanine dye Cy5.5. Precautions have to be taken when using this dye in association with APC on dual laser cytometers. It needs a cytometers capable of interlaser compensation. PE-Cy5.5 is also subject to uncoupling and photobleaching.

PE-Cy7:

Excited by 488nm argon laser. PE-Cy7 is a tandem dye using the resonance energy transfer from PE to the cyanine dye Cy7. The PE-Cy7 conjugates are just as bright as PE conjugates can be used simultaneously with many green, orange and far red fluorochromes with a minimal crossbeam compensation. Though, PE-Cy7 is particularly sensitive to photoinduced degradation. Care must be taken to avoid photobleaching.

PerCP (Peridinin Chlorophyll Protein):

Excited by 488nm argon laser. PerCP is a component of the photosynthetic apparatus found in the dinoflagellate *Glenodinium*. PerCP is a protein complex that is subject to high photobleaching. It is usually not recommended to use, since PerCP-Cy5.5, or PE-Cy5.5 are more photostable and brighter.

PerCP-Cy5.5:

Excited by 488nm argon laser. PerCp-Cy5.5 is a tandem dye using the resonance energy transfer from PerCP to the cyanine dye Cy5.5. Unlike PerCP, PerCP-Cy5.5 is not subject to photobleahching and it causes less Fc receptor-mediated nonspecific staining than PE-Cy5. It also not as susceptible to fixative or light as APC-Cy7 and PE-Cy7.

PE-Texas Red:

Excited by 488nm argon laser. PE-Texas Red is a tandem conjugate system that combines PE and Texas Red. The dye is using the resonance energy transfer from PE to Texas Red. It emits a bright fluorescence in the red region of the spectrum. Nevertheless, special care must be taken when using PE-Texas Red conjugates in conjunction with PE

since there is a considerable spectral overlap in the emission profiles of both fluorochromes.

PI (Propidium iodide):

Excited by 488nm argon laser. PI is a DNA intercalater and used as a viability dye to discriminate between viable and non viable cells.

Qdots (Qdots 525, 545, 565, 585, 605,655, 705 and 800):

Excited by 350nm UV laser or 405nm argon laser. Qdots nanocrystals are fluorophores made of a semiconductor material. The core is made of cadmium and the semiconductor shell is usually made of zinc sulfide. The brightness and photostability of Qdots outperform easily any other fluorochrome. Although they can be excited with either 350nm or 405nm lasers, it is recommended to use a 350nm laser line as Qdots have extremely high extinction coefficients when excited with a 350nm UV laser. They also have broad excitation and narrow symmetric emission properties. Filter sets must be chosen accordingly to the Qdots used since the filter selection is critical. Their small and uniform size means that their impact on normal cell function will be minimal.

RFP dTomato (Red Fluorescent Protein):

Excited by 561nm yellow–green laser. RFP is a recombinant protein that emits a bright red fluorescence in the same wavelength than PE and DS Red. Therefore, it can be used instead of DSRed. The red fluorescent protein was expressed from transformed E. coli. The recombinant RFP tomato can also be conjugated to other proteins.

RFP tdTomato (Red Fluorescent Protein):

Excited by 561nm yellow–green laser. RFP is a recombinant protein that emits an extremely bright red fluorescence in the same wavelength than PE, DS Red or RFP dTomato. The red fluorescent protein was obtained by n mutation of the RFP dTomato protein. It constitutes one of the best choice for a Tag or Report gene protein.

Texas Red:

Excited by 488nm argon laser. Texas Red emits a really bright fluorescence in the red region of the spectrum. When using Texas red and PE together, a dual-laser flow cytometer equipped with a tunable dye laser to avoid “leaking” into the PE detector is recommended. Precaution must be taken when using those two fluorochromes together for multicolor analysis.

Venus:

Excited by 488nm argon laser. Venus is a variant of the YFP protein obtained by mutagenesis. Additional mutations were also introduced in order to increase the tolerance of Venus to acidic environments and to reduce the sensitivity to chloride. The absorption and emission spectral peaks of Venus are shifted to longer wavelengths by a single nanometer compared to EYFP, but the brightness level is retained. Unfortunately, the photostability of Venus is only about 25 percent that of EYFP.

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