

7-Aminoactinomycin D

7-Aminoactinomycin D (7-AAD), like propidium iodide (PI discussed below), is a DNA intercalating dye but 7-AAD is specific for cytosine-guanine base pairs. It is well suited for viability measurements and also for apoptosis experiments where it is often paired with annexin V conjugates. During early apoptosis, phosphatidylserine is translocated from the cytoplasmic face of the plasma membrane to the external face. Annexin V, when in the presence of Ca^{2+} , has a high affinity for exposed cytoplasmic phosphatidylserine. Unlike propidium iodide, this dye has minimal emission bleed from the FL3 detector into the FL2 phycoerythrin detector. Whereas PI can be detected in either FL2 or FL3, although it is typically detected in FL2 of the FACSCalibur, 7-AAD is detected in FL3.

Allophycocyanin

Allophycocyanin (APC) is an accessory photosynthetic pigment found in blue-green algae. APC has 6 phycocyanobilin chromophores per molecule, that are similar in structure to phycoerythrobilin, the chromophore in phycoerythrin (PE). APC tandem dyes, APC-Cy5.5 and APC-Cy7, are also available. APC has a 650-nanometer wavelength absorption maximum and a 660-nanometer fluorescence emission maximum. APC can be used in flow cytometers equipped with dual lasers for multi-color analysis. Like Alexa Fluor 633, APC is excited using the helium-neon red diode laser (633 nanometers) of the FACSCalibur and is detected using the FL4 detector.

Alexa Fluor 488

Alexa Fluor 488 has a spectrum almost identical to that of fluorescein isothiocyanate (FITC), but with extraordinary photostability. Because of this photostability, it has become a choice for fluorescent microscopy applications and has become popular in cytometry applications. It is detected in the FL1 detector. Unlike other fluorochromes with similar emission spectra, Alexa Fluor 488 is pH insensitive over a broad range.

Alexa Fluor 633

Alexa Fluor 633 is a practical alternative to allophycocyanin (APC) as well as Cy5. Alexa Fluor 633 conjugates can be used in multi-color flow cytometry with instruments equipped with a second red laser or red diode. It is detected in the FL4 detector of the FACSCalibur. Like other Alexa Fluor dyes, Alexa Fluor 633 exhibits uncommon photostability, making it an ideal choice for fluorescent microscopy.

A great number of different Alexa Fluor dyes exist that are beyond the scope of this introductory fluorophore section. Many manufacturers sell directly-conjugated Alexa Fluor antibodies. [Thermo Fisher's Zenon Antibody Labeling Kits](#), which are available for all of their Alexa Fluor dyes, make it possible to rapidly and quantitatively label antibodies from a purified antibody fraction or from a crude antibody preparation, such as serum, ascites fluid or a hybridoma supernatant.

APC-Cy7

APC-Cy7 (also written Cy7-APC) is a tandem conjugate system that combines APC and a cyanine dye (Cy7) and has an absorption maximum at ~650 nanometers. This tandem conjugate uses the efficiency of the fluorescence light energy transfer between the two fluorochromes. When excited by light from a helium-neon laser, the excited fluorochrome (APC) is able to transfer its fluorescent energy to the cyanine molecule, which then fluoresces at a longer wavelength. The resulting fluorescent emission maximum is in the deep red at approximately 767 nanometers. APC-Cy7 conjugates cannot be detected on a FACSCalibur because the FL4 detector's optical filter is centered for APC emission (660 nanometers) and not the longer red emission excited with a helium-neon laser. It is recommended that special precautions be taken with this conjugate and cells stained with them to protect the fluorochrome from long-term exposure to visible light.