

Dye: Alexa Fluor 647 dye

Alexa Fluor dyes are a series of fluorescent dyes produced by Molecular Probes that span the visible spectrum. The Alexa Fluor dyes produce very bright conjugates with greater photostability and they also have well differentiated spectra providing many options for multicolour detection and many other fluorescence applications.

The Alexa Fluor 647 dye is the equivalent to fluorescent dyes such as Cy5. The Alexa Fluor 647 dye when conjugated to most proteins, oligonucleotides and nucleic acids has very little change in absorbance or fluorescence spectra hence yielding greater total fluorescence at the same degree of substitution.

Applications

Alexa Fluor dyes are typically used as cell and tissue labels in fluorescent microscopy and cell biology including western blots.

Visualization

Applications using the Alexa Fluor 647 dye can be visualized using the Syngene Diversity and G:BOX iChem range of cooled camera image capture systems. The Alexa Fluor 647 dye has an excitation and emission peak of 650 and 665nm respectively.

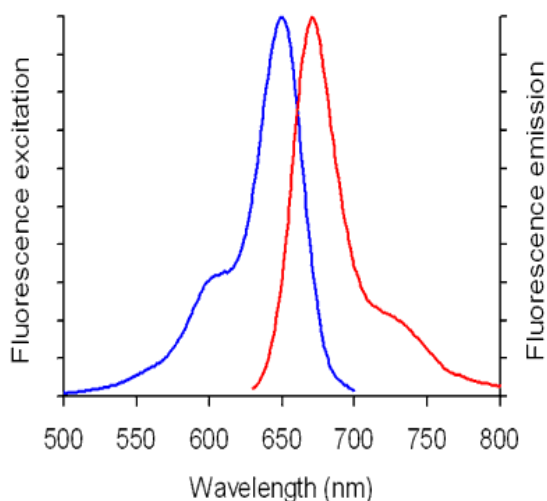


Figure 1 – Excitation (blue line) and emission (red line) spectra of Alexa Fluor 647 dye

System	Lighting	Filter
Diversity and G:BOX iChem range using cooled cameras	Epi red from RGB module Epi red from RGB module	FiltFRLP Cy5

Table 1 - Recommended lighting and filter selection for visualizing the Alexa Fluor 647 dye using Syngene image capture systems

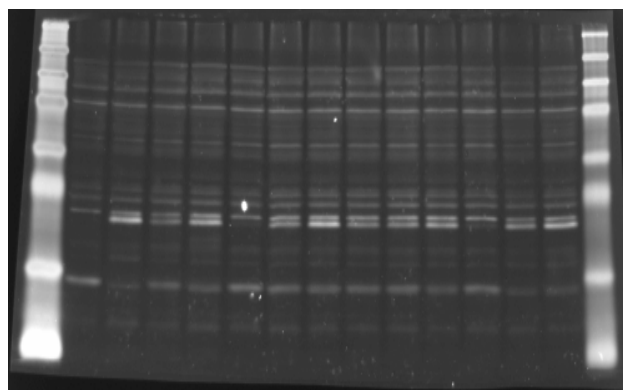


Figure 2 – Western blot visualized using the G:BOX XT image capture system

Protein samples were transferred on to a PVDF membrane and incubated with a 1/3000 dilution of the Alexa Fluor 647 goat anti-rabbit secondary antibody. Bands were visualized by exposure to Epi red light and a Cy5 filter. The image was captured using GeneSnap software (Syngene) for an exposure time of 3 minutes using the G:BOX XT Syngene image capture system.

For the ideal image using the Alexa Fluor 647 dye, the image should have good resolution, high sensitivity enabling the detection of low intensity bands and a low background improving band visibility as illustrated in the image above.

Syngene reserves the right to amend or change specifications without prior notice. This Application note supersedes all earlier versions.

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