

Secondary Antibodies for **Flow Cytometry**

For flow cytometry we offer three fluorescent proteins (**R-PE**, **APC**, and **PerCP**) conjugated to many highly adsorbed secondary antibodies, streptavidin, and purified immunoglobulin controls. The table of Secondary Antibodies for Flow Cytometry lists all of the antibodies, purified immunoglobulin controls, and streptavidin to which we conjugate these proteins. Also shown in this table are the same highly adsorbed antibodies and purified immunoglobulins conjugated to Biotin-SP and fluorescent dyes appropriate for flow cytometry (Alexa Fluor® 488, FITC, and Alexa Fluor® 647). Note that many antibodies listed elsewhere in tables of Whole IgG and F(ab')₂ Fragments also can be used for flow cytometry.

Phycoerythrin (R-PE) and **allophycocyanin (APC)** are among several kinds of light-harvesting phycobiliproteins found in red, blue-green, and cryptomonad algae. We offer R-PE, the form found in red macrophytic algae (seaweed). APC is isolated from the blue-green alga *Spirulina*, and is chemically cross-linked for stability. After phycobiliproteins are conjugated to secondary antibodies, there is little fluorescence quenching, which results in conjugates of high specific fluorescence compared with conventional fluorophore-antibody conjugates. R-PE, PerCP, and APC can be excited by light over a wide range of the visible spectrum (Figure 1), are highly water soluble, have relatively low isoelectric points, and lack potentially sticky carbohydrates.

PerCP is a fluorescent peridinin-chlorophyll-protein complex isolated from dinoflagellates. We offer the form found in *Dinophyceae* sp. with a molecular weight of about 35.5 kDa. It has a broad spectrum of excitation with a main peak at 482 nm, and a long Stokes shift to an emission peak at 677 nm (Figure 1).

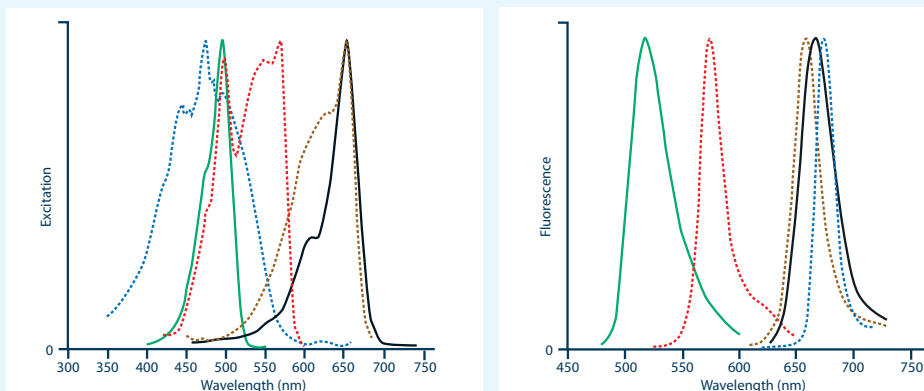


Figure 1. Excitation (left) and emission (right) spectra of Alexa Fluor® 488/FITC (green), R-PE (red), PerCP (blue), Alexa Fluor® 647 (black), and APC (brown). Peak heights were normalized after the spectra were obtained with an M-series spectrofluorometer system from Photon Technology International, Inc.

PerCP, Alexa Fluor® 488 (or FITC), and **R-PE** are excited at 488 nm with an argon laser (Figure 1), and thus can be used for one-, two-, and three-color analyses with single-laser flow cytometers. **APC** and Alexa Fluor® 647 are excited at 633 nm (Figure 1) to give a fourth color with dual-laser flow cytometers.

It should be noted that the relatively high molecular weights of **PerCP**, **R-PE**, and **APC** may preclude their use in procedures requiring good penetration into cells and tissues. They are predominantly intended for surface labeling of cells for flow cytometry.

Alexa Fluor® fluorescent dyes are a trademark of Life Technologies Corp.



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