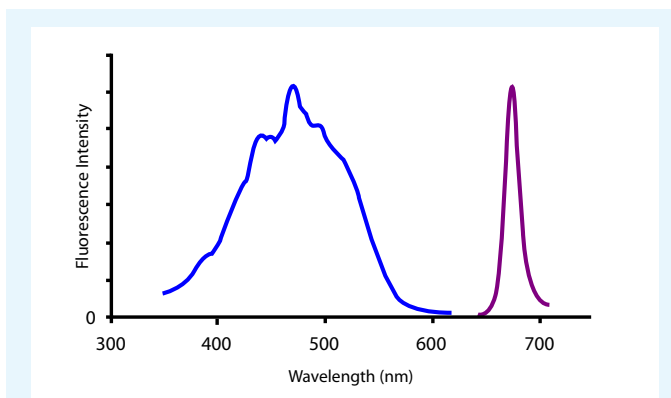


## Specializing in Secondary Antibodies and Conjugates

# PerCP Conjugates for Flow Cytometry

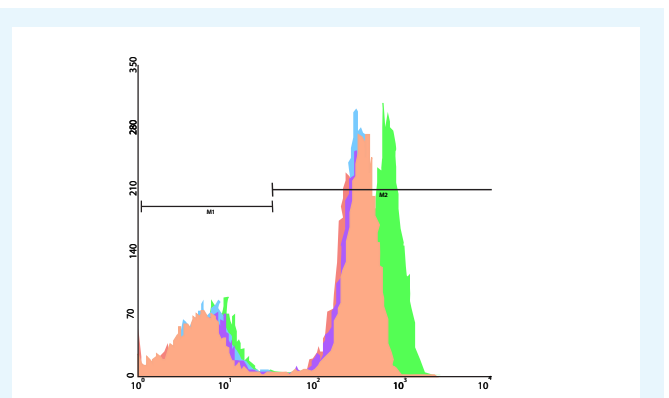
PerCP is a fluorescent peridinin-chlorophyll protein complex isolated from dinoflagellates. We offer the form found in *Dinophyceae* sp. with a molecular weight of about 35.5 kDa. It has a broad spectrum of excitation with a main peak at 472 nm, and a long Stokes shift to an emission peak at 677 nm (Figure 1).



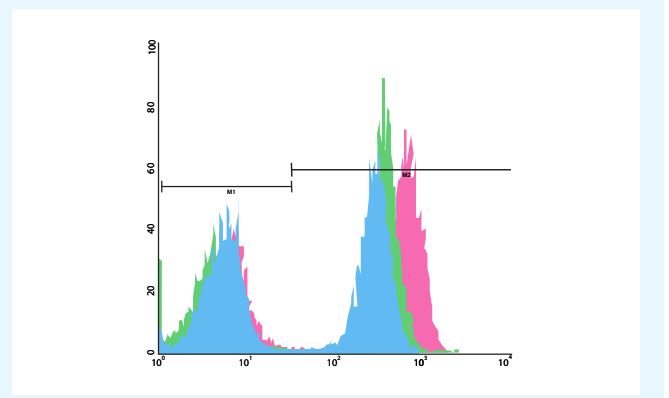
**Figure 1.** Relative shape and position of spectra in the peak region of excitation (blue) and emission (purple) for PerCP conjugated to an affinity-purified secondary antibody from Jackson ImmunoResearch. Quantitative comparisons should not be made since peak heights have been normalized. Spectra were obtained with an M-Series spectrofluorometer system from Photon Technology International, Inc.

PerCP conjugates of highly adsorbed secondary antibodies are offered to label unconjugated primary antibodies, and PerCP-streptavidin is offered to label biotinylated primary or secondary antibodies (Figures 2 and 3). Two practical labeling protocols are possible with the products.

Compared with a single-step PerCP-conjugated primary antibody (Figure 2), about the same level of fluorescence is obtained with a two-step procedure using a biotinylated primary antibody and PerCP-conjugated streptavidin. A consistent, slightly higher signal is achieved by using an unconjugated primary antibody and PerCP-conjugated secondary antibody. Although three-step procedures are usually undesirable for flow cytometry, a somewhat greater amplification may be obtained with unconjugated primary antibody, biotinylated secondary antibody, and PerCP-conjugated streptavidin (Figure 3).

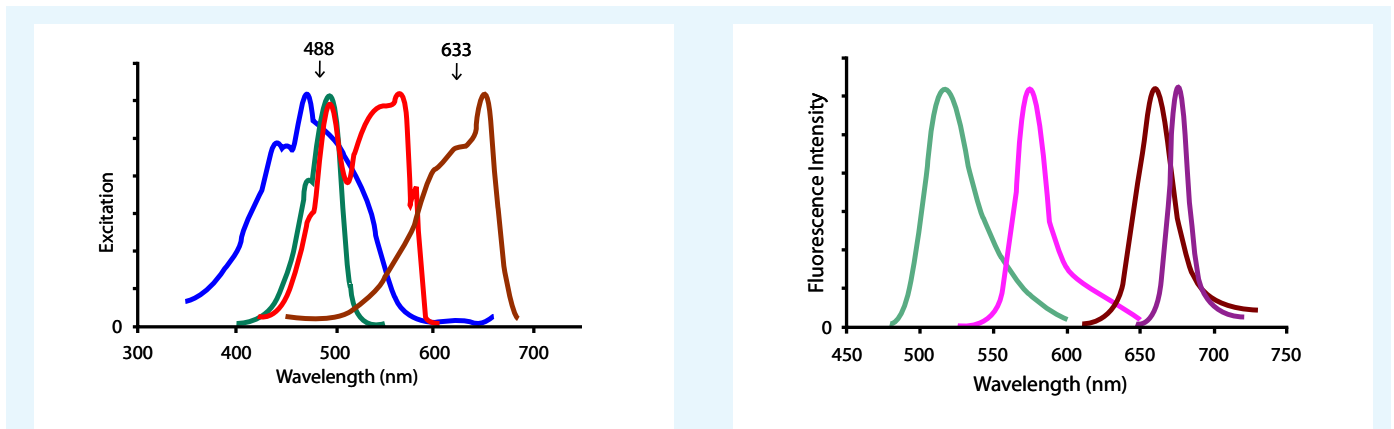


**Figure 2.** Human peripheral lymphocytes were stained either directly with PerCP-conjugated anti-CD3 (orange) from BioLegend, or indirectly with biotin-conjugated anti-CD3 (B-D Pharmingen) and PerCP-streptavidin either from Jackson ImmunoResearch (blue), BioLegend (purple), or B-D Pharmingen (red). For further comparison, cells were stained with unconjugated anti-CD3 (B-D Pharmingen) and PerCP-conjugated secondary antibody from Jackson Immuno-Research (green). Stained cells were analyzed in a dual-laser FACSCalibur flow cytometer (Becton Dickinson).



**Figure 3.** Human peripheral lymphocytes were stained with unconjugated anti-CD3 (B-D Pharmingen) and PerCP-conjugated secondary antibody from Jackson ImmunoResearch (green), or with biotin-conjugated anti-CD3 (B-D Pharmingen) and PerCP-streptavidin from Jackson ImmunoResearch (blue). For further comparison, cells were stained with unconjugated anti-CD3 (B-D Pharmingen), biotinylated secondary antibody and PerCP-streptavidin from Jackson ImmunoResearch (red). Stained cells were analyzed in a dual-laser FACSCalibur flow cytometer (Becton Dickinson).

PerCP conjugates may be used alone or with Alexa Fluor® 488 (or FITC) and R-PE for one- to three-color analyses with a single-laser flow cytometer equipped with an argon laser emitting at 488 nm. Up to four-color analyses with low compensation are easily achieved by adding APC-conjugated antibodies with 633 or 635 nm excitation provided by a dual-laser flow cytometer (Figure 4).



**Figure 4.** Excitation spectra (left) for PerCP-(blue), Alexa Fluor® 488/FITC-(green), R-PE-(red), and APC-(brown) conjugated secondary antibodies from Jackson ImmunoResearch. Emission spectra (right) for Alexa Fluor® 488/FITC-(green), R-PE-(pink), APC-(brown), and PerCP-(purple) conjugated secondary antibodies from Jackson ImmunoResearch. Quantitative comparisons should not be made since peak heights have been normalized. All spectra were obtained with an M-Series spectrofluorometer system from Photon Technology International, Inc.



Certified by BSI to ISO 9001:2008 under certificate number FM 545248.

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