Phycobiliproteins and Their Fluorescent Labeling Applications

The phycobiliproteins are composed of a number of subunits, each having a protein backbone to which linear tetrapyrrole chromophores are covalently bound. Phycoerythrins (red) and phycocyanins (blue) are the two major classes of phycobiliproteins. The Absorption maxima for phycoerythrins (PE) lie between 490 and 570 nm while absorption maxima for phycocyanins (PC) are found between 610 and 665 nm. In general, phycobiliproteins have good long-term stability when stored refrigerated as ammonium sulfate precipitates. Purified biliproteins may disassociate into subunits under acidic or basic conditions, but are relatively stable at room temperature at neutral pH with concentrations greater than 0.1 mg/mL. Disassociated subunits typically have less intense coloration and fluorescence than the native pigment. *It is recommended that all phycobiliproteins and their conjugates (preferably in neutral buffer solution) be refrigerated, never frozen.*

The phycobiliproteins [including B-phycoerythrin (B-PE), R-phycoerythrin (R-PE) and allophycocyanin (APC)] are ultra-sensitive fluorescent dyes for biological detections. They are >100 times more sensitive than conventional organic fluorophores. Even in practical applications such as flow cytometry and immunoassays, the sensitivity of phycobiliprotein-conjugated antibodies is usually much greater than that of the corresponding organic molecule-based conjugate. Phycobiliproteins, the brightest fluorescent tags, have multiple sites for forming stable conjugation to many biological and synthetic materials.

B-Phycoerythrin (B-PE) has three absorption bands with maximum absorption at 545 nm. The subunit structure of B-PE is similar to that of R-PE, but the chromophore content of the subunits differs, causing the difference in the relative intensities of the absorption peaks: α and β subunits contain only PEB while γ subunit contains PEB and PUB. B-PE is found both in cyanobacteria and red algae. The intense pink color and orange fluorescence of B-PE are virtually indistinguishable from those of R-PE by naked eyes. Allophycocyanin is the least stable among the major phycobiliproteins, susceptible to dissociation at low concentrations including concentrations at which some assays are performed. For this reason, many researchers prefer to use CL-APC which is chemically cross-linked between α and β subunits and much more stable than APC.

R-Phycoerythrin (R-PE) is isolated from red algae. Its primary absorption peak is at 565 nm with secondary peaks at 496 nm and 545 nm. The relative prominence of the secondary peaks varies significantly among R-PEs from different species. R-PE has three types of subunits: α (~20,000 daltons), β (~20,000 daltons) and γ (~30,000 daltons). The molecular weight of intact R-PE has been found to be about 240,000 daltons, and a subunit structure of $(\alpha\beta)_6\gamma$ has been determined. The α subunit of R-PE contains only the phycoerythrobilin (PEB) chromophore, while β and γ subunits contain both PEB and phycourobilin (PUB). Variability in the absorption spectra of R-PEs from various species reflects differences in the PEB/PUB ratio of the subunits. R-PE and closely related B-PE are the most intensely fluorescent phycobiliproteins, with quantum efficiencies probably in excess of 90%, and its orange fluorescence is readily visible by eye in any moderately concentrated solution.

C-Phycocyanin (C-PC) occurs as the major phycobiliprotein in many cyanobacteria and as a secondary phycobiliprotein in some red algae. The pigment has a single visible absorption maximum between 615 and 620 nm and a fluorescence emission maximum at ~650 nm. Its molecular weight is between 70,000 and 110,000 daltons. The pigment is composed of two subunits, α and β, which occur in equal numbers, but the exact number of α and β pairs which make up the molecule may vary among the species. Both α and β subunits contain only the PCB chromophore. In addition to absorbing light directly, this intensely blue pigment accepts quanta from phycoerythrin by fluorescent energy transfer in organisms in which PE is present. The red fluorescence of C-PC is transferred to allophycocyanin.

Cat. #	Name	Size	MW	Ex (nm)	Em (nm)	QY*
2601	B-PE (B-Phycoerythrin)	1 mg	~240,000	545	575	0.98
2552	CL-APC (Cross Linked-Allophycocyanin)	1 mg	~105,000	651	662	0.68
2549	CL-APC (Cross Linked-Allophycocyanin)	10 mg	~105,000	651	662	0.68
2550	CL-APC (Cross Linked-Allophycocyanin)	50 mg	~105,000	651	662	0.68
2551	CL-APC (Cross Linked-Allophycocyanin)	100 mg	~105,000	651	662	0.68
2553	C-PC (C-Phycocyanin)	1 mg	~264,000	616	647	0.81

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2554	APC (Allophycocyanin)	1 mg	~105,000	651	662	0.68
2558	R-PE (R-Phycoerythrin)	1 mg	~240,000	565	575	0.84
2556	R-PE (R-Phycoerythrin)	10 mg	~240,000	565	575	0.84
2557	R-PE (R-Phycoerythrin)	100 mg	~240,000	565	575	0.84
2559	PerCP (Peridinin-Chlorophyll-Protein Complex)	1 mg	~35,000	482	677	1
2540	PerCP (Peridinin-Chlorophyll-Protein Complex)	10 mg	~35,000	482	677	1

* QY = fluorescence quantum yield