

PHYCOLINK[®] ACTIVATED APC

SPECIFICATIONS

Product Code: PJ25C
A₆₅₀/A₂₈₀: ≥4.5
Concentration: 9.5 - 10.5 mg/ml
Conjugation Performance: ≥50% incorporated in a standard antibody conjugation.
 Shipped with cold pack for next day delivery.
 Store at 4°C (dark) **DO NOT FREEZE**
Formulation: Supplied in 50 mM MES, 2 mM EDTA (pH 6.0) with 1 µg/ml pentachlorophenol.
Stability: Stable at least 6 months when stored properly.

ProZyme[®] PhycoLink[®] SMCC-Activated Cross-linked Allophycocyanin (Activated APC) is a stabilized form of the highly fluorescent phycobiliprotein Allophycocyanin (APC), which has been chemically activated for easy conjugation to immunoglobulin molecules or other sulfhydryl-containing proteins. The sulfhydryl-reactive group is maleimide and readily reacts with free cysteine residues under mild conditions of temperature and pH.

PhycoPro[™] PB25 Cross-linked APC is treated with SMCC under conditions that result in only a few SMCC modifications to each protein. This derivatized protein has been purified to remove excess SMCC. It is stable for extended periods of time;

SMCC-activated phycobiliproteins have been stored in MES buffer for up to 1 year at 4°C while retaining conjugation reactivity.

Cross-linking for Stability

Upon dilution or exposure to chaotropic salts, APC reversibly disassociates. One molar sodium perchlorate has been shown to be a particularly effective agent for demonstrating the disruption of the APC quaternary structure. This change in structure causes changes in both the absorption and emission spectra. In particular, the characteristic absorption peak at 650 nm is lost (Fig 1).

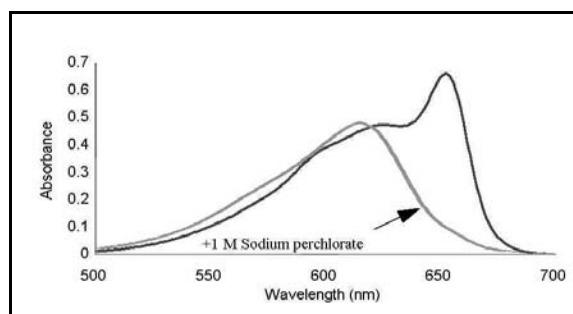


Fig 1 – Allophycocyanin

APC decomposition can be prevented by the introduction of crosslinks between α and β subunits. Fig 2 shows the increased stability of PhycoPro Cross-linked APC in the presence of a chaotropic salt compared to APC. Table 1 summarizes the values measured for the primary and secondary peaks with and without treatment and reports the ratio. These data clearly demonstrate the utility of cross-linking for maintaining the structural integrity of APC.

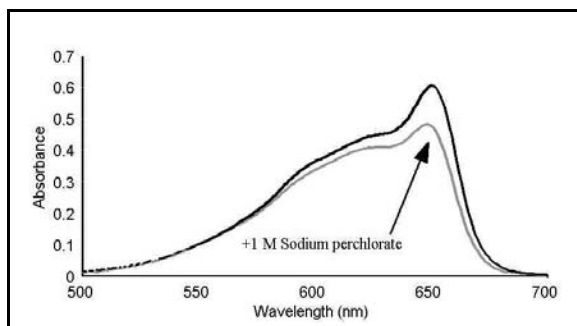


Fig 2 – ProZyme Cross-linked Allophycocyanin

Protein	sp	A ₆₁₅	A ₆₅₀	ratio
PhycoPro GT5™ APC	–	0.45	0.65	1.43
	+	0.48	0.11	0.24
PhycoPro Cross-linked APC	–	0.42	0.61	1.44
	+	0.39	0.48	1.22

Table 1 - Absorbance differences before and after treatment of APC and Cross-linked APC with sodium perchlorate (sp).

For additional information about APC, please request ProZyme's Technical Data Sheet for PB25 Cross-linked Allophycocyanin or PB22 GT5™ Allophycocyanin.

Purity and Brightness

The ratio of absorbance at the wavelength of maximum absorbance to absorbance at 280 nm ($A_{\lambda_{max}}:A_{280}$) is indicative of the purity of the APC with respect to most forms of contaminating protein. A_{280} is primarily due to aromatic amino acids, and this is roughly proportional to the total concentration of protein in solution, including APC. A_{650} (λ_{max} for Activated APC) reflects only the concentration of APC. However, $A_{650}:A_{280}$ also indicates the condition of the phycobiliprotein itself, since highly purified samples of APC can vary significantly in this respect. These differences reflect handling, age and the source of the original material; reductions in this ratio probably reflect deterioration of chromophores.

ProZyme uses only very high quality APC providing exceptional brightness which remains throughout subsequent processing to give the greatest sensitivity to antibody conjugates.

Cross-linked APC is emerging as one of the preferred fluorescent probes for Fluorescence Activated Cell Sorting (FACS) and Förster-type Fluorescence Resonance Energy Transfer (FRET) assays. APC is a protein of approximately 100 kD molecular weight containing many fluorophores. Unlike small molecule dyes, typically, about one APC molecule is conjugated per molecule of antibody. Nonetheless, by virtue of its high absorption coefficient, almost perfect quantum efficiency and emission in a region of the spectrum with extremely low cellular autofluorescence, it is one of the brightest dyes available today. It emits at about 650 nm, and can be excited by either a dye laser (595-605 nm), or the less expensive HeNe laser (633 nm).

CHARACTERISTICS

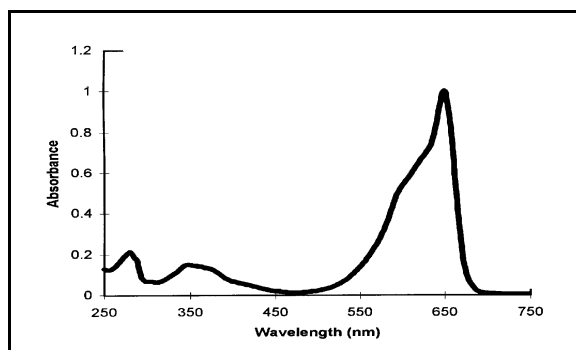
A_{650}/A_{280} : ≥ 4.5

Extinction coefficient: $E_{650}^{1\%} = 70$

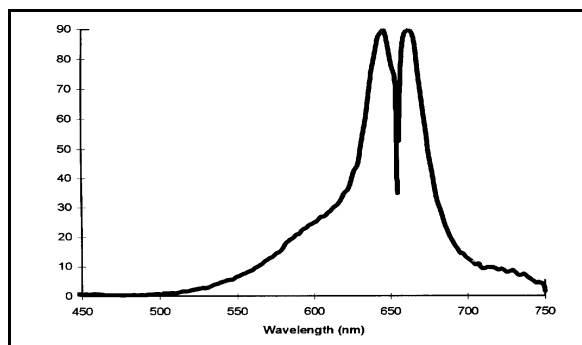
Absorption maximum: 650 nm

Emission maximum: 658 nm

Absorbance Spectrum:



Fluorescence Spectra (arbitrary units normalized to absorbance, excitation 450–655 nm, emission 655–750 nm):



REFERENCES

Glazer, A. N. Phycobilisomes: structures and dynamics. **Ann Rev Microbiol** **36**: 173–198 (1982).

Hardy, R. R. Purification and coupling of fluorescent proteins for use in flow cytometry. In: **Handbook of Experimental Immunology**, 31.1–31.12, 4th ed. (D. M. Weir, L. A. Herzenberg, C. Blackwell and L. A. Herzenberg, eds) Blackwell Scientific Publications, Boston (1986).

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MacColl, R. and D. Guard-Friar. **Phycobiliproteins**. CRC Press, Inc., Boca Raton, Florida (1987).

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