PROzyme®

NOTICE: ProZyme was purchased by Agilent in July 2018. Documents for products and product lots manufactured before August 2019 will contain references to ProZyme. For more information about these products and support, go to: **www.agilent.com/en/contact-us.**

Agilent

GLYKO®

CROSS-LINKED ALLOPHYCOCYANIN

SPECIFICATIONS

Product Code: PB25
Concentration: ≥10.0 mg/ml
Purity: A_{650}/A_{280} ≥4.60
A_{650}/A_{620} ≥1.50
Cross-link Ratio: ≥1.00
Produced from PB22 GT5™ Allophycocyanin. Shipped with ice pack for next day delivery.
Store at 4°C in the dark. **DO NOT FREEZE.**
Formulation: Protein is supplied as a suspension in 0.1 *M* phosphate buffer with 60% ammonium sulfate.
Stability: Stable at least 12 months when stored properly.

Several forms of allophycocyanin (APC) have been identified, depending on both the organism studied and the exact function of the individual molecule in the final transfer of energy from the phycobilisome to the chlorophyll reaction center. The most common form has an absorbance maximum at about 650 nm. Like C-phycocyanin (CPC), APC carries only the phycocyanobilin (PCB) chromophore; its significantly different spectral properties result solely from conformation effects on the chromophores. It possesses α and β subunits with an apparent $(\alpha\beta)_3$ quaternary structure, and is bright blue to the eye.

Like other phycobiliproteins, PhycoPro™ PB25 Cross-linked APC is fluorescent, with an extremely high absorbtivity, a high quantum efficiency and excitation and emission peaks at visible wavelengths. It can be easily linked to antibodies and other proteins by conventional protein cross-linking techniques without altering its spectral characteristics.

Upon dilution or exposure to chaotropic salts, APC reversibly dissociates. 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 (Figure 1).

APC decomposition can be prevented by the introduction of specific crosslinks between αβ subunits. Figure 2 shows the increased stability of PB25 Cross-linked APC in the presence of a chaotropic salt, sodium perchlorate, when compared to APC.

Table 1 summarizes measurements for the primary and secondary peaks of both GT5™ APC (ProZyme product code PB22) and PB25 Cross-linked APC, with and without perchlorate treatment, and reports the A_{650}/A_{620} ratio. The cross-link ratio (defined as the A_{650}/A_{620} ratio obtained in the presence of 1 *M* sodium perchlorate) is a measure of the effectiveness of cross-linking. These data clearly demonstrate the utility of crosslinking for maintaining the structural integrity of APC.

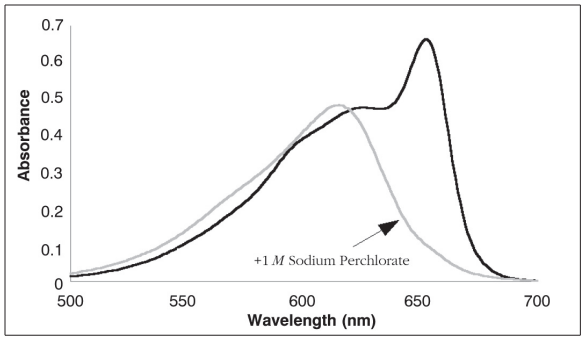


Figure 1 – GT5™ Allophycocyanin

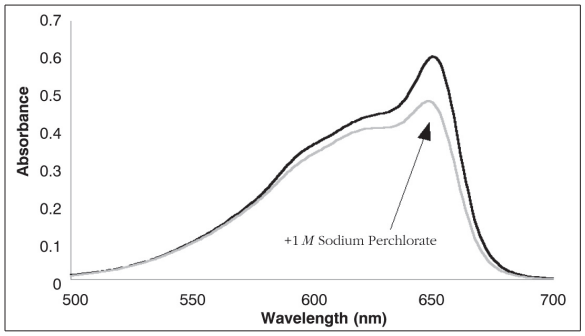


Figure 2 – Cross-linked Allophycocyanin

Source	P	A_{650}	A_{620}	Ratio
GT5™ APC	–	1.19	0.73	1.64
	+	0.25	0.79	0.31
Cross-linked APC	–	0.80	0.51	1.56
	+	0.66	0.50	**1.31**

Table 1 - Comparison of absorbance ratios with and without treatment of GT5™ APC and Cross-linked APC with 1 *M* sodium perchlorate (P).

The cross-link ratio (shown in bold in Table 1) is obtained by diluting 0.1 - 0.15 mg of cross-linked APC into 1 ml of 50 m*M* Tris-HCl, 1 *M* sodium perchlorate (pH 7.7). Absorbance measurements are taken after 30 minutes at room temperature.

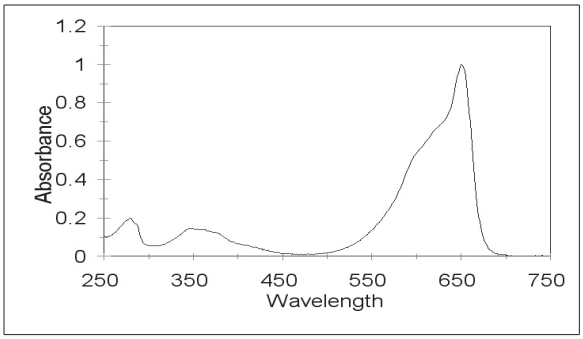
CHARACTERISTICS

Cross-link Ratio: $A_{650}/A_{620} \geq 1.0$

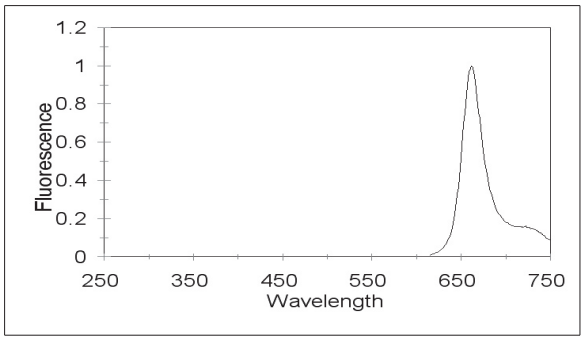
Molecular Weight: 104,000 daltons

Extinction Coefficient: $E^{1\%}_{650} = 73$

Absorbance Spectrum:



Fluorescence Emission Spectrum:
(arbitrary units; excitation at 610 nm)



Purity: The purity of the APC was measured following crosslinking:

$A_{650}/A_{280} \geq 4.60$
$A_{650}/A_{620} \geq 1.50$

A_{650}/A_{280} is indicative of the purity of the preparation with respect to most forms of contaminating protein. Absorbance at 280 nm is primarily due to aromatic amino acids, and thus is roughly proportional to the overall concentration of protein in solution, including APC. Absorbance at 650 nm reflects only the concentration of APC.

Spirulina produces both APC and CPC (absorption maximum at 620 nm). An $A_{650}/A_{620} \geq 1.5$ indicates that the APC is not significantly contaminated with CPC.

Origin: USA

REFERENCES

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Kronick, M. N. The use of phycobiliproteins as fluorescent labels in immunoassay. **J Imm Meth 92:** 1 - 13 (1986).

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

MacColl, R., K. Csatorday, D. Berns and E. Traeger. The relationship of the quaternary structure of allophycocyanin to its spectrum. **Arch Biochem Biophys 208(1):** 42 - 48 (1981).

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