

# HASH BIOTECH LIMITED

A GROUP COMPANY OF HASH GROUP



## ALLOPHYCOCYANIN

### CHARACTERISTICS

MOLECULAR WEIGHT	: 104KDa
COMPOSITION	: ALLOPHYCOCYANIN COMPRISES OF TWO SUBUNITS $\alpha$ AND $\beta$ AND IS PRESENT AS TRIMER ( $\alpha\beta$ ) <sub>3</sub> . MOLECULAR WEIGHT OF THE RANGE OF 17KDA FOR $\alpha$ -SUBUNIT AND 31.5 KDA FOR $\beta$ -SUBUNIT.
PURITY	: $A_{651}/A_{280} \geq 4$ , $A_{651}/A_{620} > 1.4$
ABSORPTION MAXIMA	: 648-652 nm
EMISSION MAXIMA	: 660 nm
EXTINCTION COEFFICIENT	: E1% AT 620 = 73
ORIGIN	: India
SOURCE	: <i>Spirulina platensis</i>

ALLOPHYCOCYANIN (APC) IS A BLUE GREEN PROTEIN COMPLEX OCCURRING IN CYANOBACTERIAL PHYCOBILISOMES WITH HIGH FLUORESCENCE QUANTUM YIELD, MOLAR EXTINCTION COEFFICIENTS, ABSORPTIVITY AND STABILITY. IT CAN BE EASILY CROSS LINKED WITH ANTIBODIES AND OTHER PROTEINS BY USING CONVENTIONAL MOLECULAR TAGGING TECHNIQUES WITHOUT LOSING ITS FLUORESCENT PROPERTIES. IT IS AN OLIGOMERIC PROTEIN WITH THE LINEAR TETRAPYRROLE CHROMOPHORES KNOWN AS BILINS COVALENTLY ATTACHED TO THE APOPROTEIN BY A THIOETHER LINKAGE[1].

### PROPERTIES OF ALLOPHYCOCYANIN:

#### ► HIGH EXTINCTION COEFFICIENTS AND QUANTUM YIELD:

ALLOPHYCOCYANIN BASED DYES EXHIBIT INTENSE FLUORESCENCE LIGHT EMISSION AND OPTIMAL BRIGHTNESS DUE TO HIGH EXTINCTION COEFFICIENTS AND QUANTUM YIELD VALUES OF THE DYES - INTENSITY INCREASES WHEN COUPLED TO BIOMOLECULES RESULTING IN REDUCED INFLUENCE OF UNCOUPLED DYE.

#### ► HIGH RESISTANCE TO PHOTO-BLEACHING:

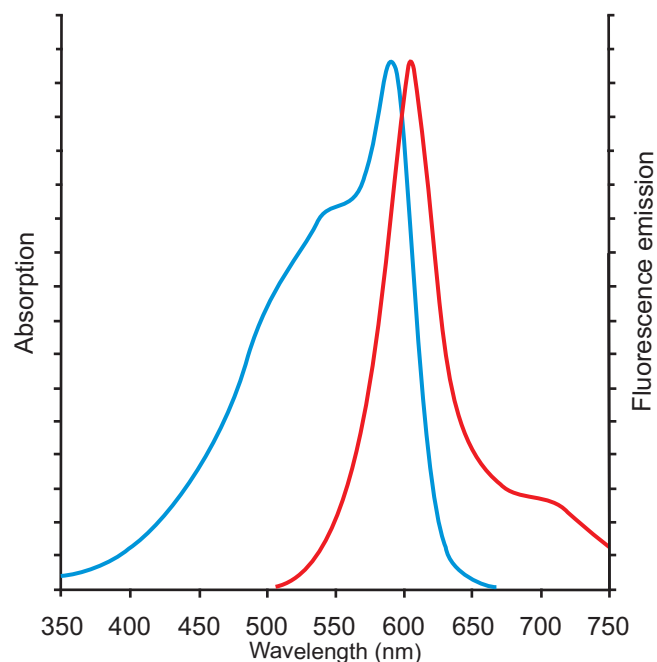
AS COMPARED TO OTHER FLUORESCENT DYES, PHYCOBILIPROTEIN BASED DYES (LIKE ALLOPHYCOCYANIN) CAN WITHSTAND HIGHER PHOTO-DEGRADATION, HENCE THEY SHOW LESSER PHOTO-BLEACHING EFFECT.

#### ► PH STABILITY:

PHYCOBILIPROTEIN BASED DYES ARE STABLE AT A WIDER RANGE OF PH AS COMPARED TO OTHER FLUORESCENT DYES (PH RANGE- 5 TO 8).

#### ► HYDROPHILIC IN NATURE:

THE NEGATIVELY CHARGED ALLOPHYCOCYANIN BASED DYES ARE HIGHLY WATER SOLUBLE AND THUS CAN BE USED IN BIOLOGICAL TEST DONE IN VITRO AND IN VIVO.



Absorption and fluorescence emission spectra of allophycocyanin in pH 7.5 buffer

### ADVANTAGES OF USING ALLOPHYCOCYANIN BASED DYES:

- HIGHLY STABLE
- HYDROPHILIC IN NATURE
- HIGH QUANTUM EFFICIENCY
- EASILY LINKED TO BIOMOLECULES LIKE ANTIBODIES
- LARGE STOKES SHIFTS - EXCITATION AND EMISSION BANDS AT VISIBLE WAVELENGTHS
- INTENSE LONG WAVELENGTH EXCITATION AND EMISSION (RELATIVELY FREE OF INTERFERENCE FROM OTHER BIOLOGICAL MATERIALS).
- FLUORESCENCE NOT QUENCHED BY EXTERNAL AGENTS (THE FLUOROPHORES ARE PROTECTED BY COVALENT BINDING TO THE PROTEIN BACKBONE).

### APPLICATIONS:

#### ► BIOLOGICAL MARKERS:

KNOWN FOR THESE UNIQUE CHARACTERISTICS, PHYCOBILIPROTEINS ARE WIDELY USED IN THE INDUSTRIAL, CLINICAL, OR RESEARCH IMMUNOLOGY LABORATORIES AS LABELS FOR ANTIBODIES, RECEPTORS AND OTHER BIOLOGICAL MOLECULES. ALLOPHYCOCYANIN (APC) IS



EMERGING AS ONE OF THE BRIGHTEST FLUORESCENT PROBES FOR FACS ANALYSIS. TYPICALLY, ONLY ONE APC MOLECULE IS CONJUGATED TO AN ANTIBODY. NONETHELESS, BY VIRTUE OF ITS HUGE ABSORPTION COEFFICIENT AND ALMOST PERFECT QUANTUM EFFICIENCY, AS WELL AS EMISSION IN A REGION OF THE SPECTRUM WITH EXTREMELY LOW CELLULAR AUTO-FLUORESCENCE, IT IS ONE OF THE BRIGHTEST DYES USED TODAY. IT EMITS AT ABOUT 680 NM, AND CAN BE EXCITED BY EITHER A DYE LASER (595-605 NM), OR THE CHEAPER HE NE LASER (633 NM).

ALLOPHYCOCYANIN HAS A GREAT UTILITY IN BIOMARKERS BECAUSE OF ITS HIGH FLUORESCENCE, BUT TYPICALLY IN COMBINATION WITH R-PE TO FORM A FLUORESCENT TANDEM PHYCOBILIPROTEIN CONJUGATE WHICH IMPROVES SENSITIVITY[2][3].

► PHARMACOLOGICALLY ACTIVE COMPOUND:

ALLOPHYCOCYANIN IS APPLIED IN HEALTH CARE PRODUCTS DUE TO CERTAIN FUNCTIONAL EFFECTS SUCH AS INHIBITION OF ENTEROVIRUS 71- INDUCED APOPTOSIS. AT CONCENTRATION NONTOXIC TO THE HOST CELLS ( $0.045 \pm 0.012 \mu\text{M}$ ), ALLOPHYCOCYANIN WAS FOUND TO INHIBIT ENTEROVIRUS 71-INDUCED CYTOPATHIC EFFECTS, VIRAL PLAQUE FORMATION, AND VIRAL-INDUCED APOPTOSIS [4][5].

► COLORANT:

IT USED AS A FOOD COLORANT BECAUSE OF ITS ALGAL BASED SOURCE, HYDROPHILIC NATURE AND STABILITY AT ROOM TEMPERATURE.

REFERENCES:

- [1] GANTT, E.; LIPSCHUTZ, C.A. (1973). ENERGY TRANSFER IN PHYCOBILISOMES FROM PHYCOERYTHRIN TO ALLOPHYCOCYANIN. *BIOCHIM BIOPHYS ACTA* 292:858-861.
- [2] MORSEMAN, J.P.; MOSS, M.W. (2001). HIGH FLUORESCENT INTENSITY CROSS-LINKED ALLOPHYCOCYANIN. WO PATENT 0196383, 2 DECEMBER 2001.
- [3] ROEDERER, M. (1998). CY7-ALLOPHYCOCYANIN CONJUGATES FOR USE IN MULTIPLEX FLUORESCENCE DETECTION ASSAYS. US PATENT 5,714,386, 3 FEBRUARY 1998.
- [4] CHUEH, C.C. (2002). METHOD OF ALLOPHYCOCYANIN INHIBITION OF ENTEROVIRUS AND INFLUENZA VIRUS REPRODUCTION RESULTING IN CYTOPATHIC EFFECT. US PATENT 6,346,408, 12 FEBRUARY 2002.
- [5] SHIH, S.R.; TSAI, K.N.; LI, Y.S. (2003). INHIBITION OF ENTEROVIRUS 71- INDUCED APOPTOSIS BY ALLOPHYCOCYANIN ISOLATED FROM A BLUE GREEN ALGA SPIRULINA PLATENSIS. *J MED VIROL* 70:119-125.

