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

PROzyme INC.

R-PHYCOERYTHRIN (PB31)

SPECIFICATIONS

Catalog No: PB31

Purity: $A_{566}/A_{280} > 5.30$
$A_{566}/A_{496} < 1.5$
$A_{620}/A_{566} < 0.005$
>98% stained protein by native polyacrylamide gel electrophoresis (Ornstein-Davis).
Absorbance and fluorescence emission spectra are reported on the Certificate of Analysis.

Functional Integrity: RQY > 2.50
Shipped on ice pack for next day delivery. Store at 4°C in the dark. DO NOT FREEZE.

Protein Concentration: >10 mg/ml

Stability: Protein is supplied as a 60% Ammonium Sulfate suspension in 50 mM phosphate buffer and 5 mM Sodium Azide as a preservative, and is stable for at least 12 months when stored properly.

R-Phycoerythrin (RPE) was originally isolated from red algae and has not been found in other taxa. Its primary absorbance peak occurs at 566 nm with secondary peaks at 496 and 545 nm; the relative prominence of the secondary peaks varies significantly among RPEs from different species. RPE has three types of subunits: α (~20,000 daltons), β (~20,000 daltons) and γ (~30,000 daltons). The molecular weight of intact RPE 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 RPE contains only the phycoerythrobilin (PEB) chromophore, while the β and γ subunits contain both PEB and phycourobilin (PUB). Variability in the absorbance spectra of RPEs from various species reflects differences in the PEB:PUB ratio of the subunits. RPE and closely related BPE are the most intensely fluorescent of the phycobiliproteins, with quantum efficiencies probably in excess of 90%, and its orange fluorescence is readily visible by eye in any moderately concentrated solution.

Because of their properties, phycobiliproteins have been used in a variety of immunological assays and as fluorescent labels for cell-sorting and homogeneous time-resolved fluorescence. In addition, because of the high molar absorbtivity of these proteins at visible wavelengths, they are convenient markers in such applications as gel electrophoresis, isoelectric focusing and gel exclusion chromatography.

ProZyme® RPE is a phycobiliprotein isolated from red algae developed as a source of choice because it yields one of the most highly fluorescent of the RPEs. Like other phycobiliproteins, ProZyme RPE is fluorescent, with an extremely high absorbtivity, a high quantum efficiency, a large Stokes shift and excitation and emission

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bands at visible wavelengths. It is a stable protein which can be easily linked to antibodies and other proteins by conventional protein cross-linking techniques without altering its spectral characteristics.

In seaweed from natural sources or seaweed farms, proteases become active within minutes or hours of harvest, and can cause complete or partial degradation of phycobiliproteins. One very typical result of partial degradation is a lowering of the A_{566}/A_{280} ratio. As a result, when seaweed that has been harvested and then stored— even if it is stored frozen— the finished RPE can have an intrinsically lower A_{566}/A_{280} ratio, which cannot be increased even through exhaustive purification. (This is one problem with the A_{566}/A_{280} ratio as an indicator of purity: it is an indicator of the condition of the pigment as well as an indicator of degree of purification.) When an acceptable reading is obtained, it indicates that the protein is both pure and in good condition, but when lower values are obtained it is not immediately clear whether the problem is in purification or pigment condition.

ProZyme RPE is made from seaweed cultured in the laboratory to control growth conditions and nutrition, and to avoid contamination from extraneous organisms and wastes found in the open ocean. It is harvested at the optimal stage of the growth cycle to assure uniform product characteristics. The pigment is extracted and stabilized within minutes of harvest, virtually eliminating risks from the action of proteases.

CHARACTERISTICS

Molecular weight: 240,000 daltons

Composition: The protein has an $(\alpha\beta)_6\gamma$ composition. Both α- and β-subunits are approximately 20,000 daltons, and the γ-subunits approximately 30,000 daltons.

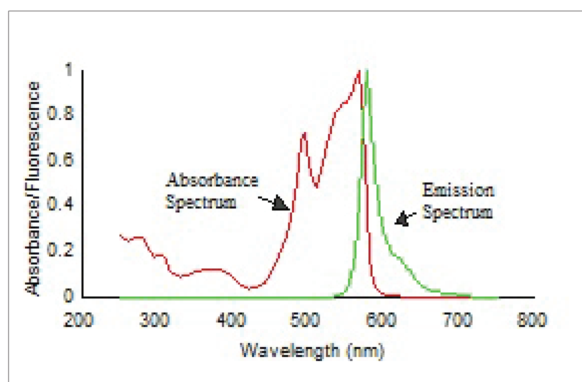
Purity: $A_{566}/A_{280} > 5.30$
$A_{566}/A_{496} < 1.5$
$A_{620}/A_{566} < 0.005$

A_{566}/A_{280} is indicative of the purity of the preparation with respect to most forms of contaminating protein. Absorbance at 280 nm in these preparations is primarily due to aromatic amino acids, and thus is roughly proportional to the overall concentration of protein in solution, including RPE. Absorbance at 566 nm reflects only the concentration of RPE.

A_{566}/A_{496} is indicative of the identity of the purified pigment; RPE has a strong secondary absorbance peak at 496 nm, where B-Phycoerythrin (BPE) exhibits only a slight shoulder. An $A_{566}/A_{496} < 1.5$ occurs only when a strong secondary peak is present, indicating that the pigment is RPE, and not significantly contaminated with BPE.

A_{620}/A_{566} is a rough indicator of the level of contamination with R-phycocyanin, although RPE exhibits a slight residual absorbance at 620 nm.

Purity measurements by native polyacrylamide gel electrophoresis (PAGE) assess the abundance of individual contaminating proteins. Gels are run with a 4% stacking gel and a 7.5% running gel. The limit of detection for contaminating protein with these gels is about 0.5% of the main band. ProZyme RPE has no significant contaminants by gel electrophoresis.



Absorbance maximum: 566 nm

Emission maximum: 575 nm

Extinction coefficient: $E_{566}^{1\%} = 82$

Functional integrity: Relative quantum yield (RQY) is an indicator of the efficiency with which absorbed quanta are reradiated as fluorescence by RPE, normalized to the quantum efficiency of a standard compound, rhodamine 504 (chosen because it absorbs and fluoresces in the same wavelength). Passing values (>2.50) indicate that the pigment is functionally intact.

Origin: USA

REFERENCES

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

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

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