

PHYCOLINK[®] ACTIVATED RPE

SPECIFICATIONS

Product Code: PJRC10
A₅₆₆/A₂₈₀: ≥5.0
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 continually strives to offer the highest quality materials. With PJRC10, the activation reaction has been optimized to yield an activated R-Phycoerythrin that produces even brighter conjugations and lower background.

ProZyme[®] PhycoLink[®] SMCC-Activated R-Phycoerythrin (Activated RPE) is a highly fluorescent phycobiliprotein, 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[™] RPE 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.

Background

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 phycoerythrobilin (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.

RPE has been used in a variety of immunological assays and as a fluorescent label for Fluorescence Activated Cell Sorting (FACS). Unlike small molecule dyes, typically about one R-phycoerythrin molecule is conjugated per molecule of antibody. Nonetheless, by virtue of its high absorption coefficient and almost perfect quantum efficiency, it is one of the brightest dyes available today. It emits at about 575 nm, and can be excited by common argon laser lines. In addition, because of the high molar absorptivity of these proteins at visible wavelengths, they are convenient markers in such applications as gel electrophoresis, isoelectric focusing and gel exclusion

chromatography.

Purity and Brightness

The ratio of absorbance at the wavelength of maximum absorbance to absorbance at 280 nm ($A_{\lambda_{\max}}:A_{280}$) is often used as a product specification; it is indicative of the purity of the RPE 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 RPE. A_{566} (λ_{\max} for Activated RPE) reflects only the concentration of RPE. However, $A_{566}:A_{280}$ also indicates the condition of the phycobiliprotein itself, since highly purified samples of RPE 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.

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.

PhycoPro RPE is isolated from a red alga developed as a source of choice because it yields one of the most highly fluorescent of the RPEs. It is 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 carefully tended and 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.

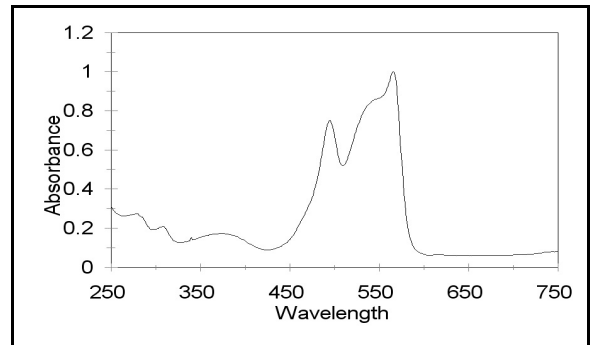
ProZyme uses only this very high quality RPE, providing exceptional brightness which remains throughout subsequent processing to give the greatest sensitivity to antibody conjugates.

For further information about this or other phycobiliproteins, visit ProZyme's webpage at www.prozyme.com.

CHARACTERISTICS

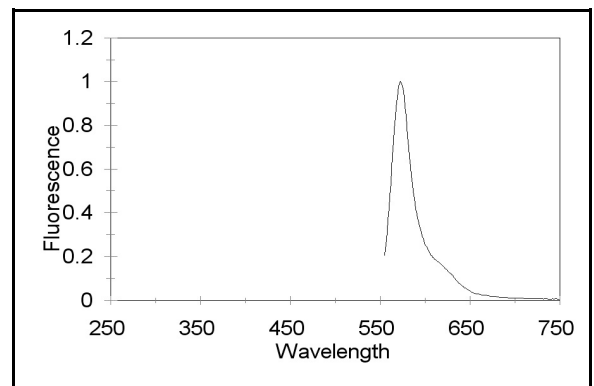
| | |
|--------------------------------------|----------------------|
| A_{566}/A_{280}: | ≥ 5.0 |
| Extinction coefficient: | $E_{566}^{1\%} = 82$ |
| Absorption maximum: | 566 nm |
| Excitation maximum: | 565 nm |
| Emission maximum: | 575 nm |

Absorbance Spectrum:



Fluorescence Emission Spectrum

(arbitrary units, excitation at 545 nm):



REFERENCES

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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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