Product Description

NUMBER | DESCRIPTION
-------|-------------
46180  | Allophycocyanin, 2 mg
46185  | R-Phyceroerythrin, 2 mg

Store these products at 4°C, protected from light. Do not freeze. Supplied as an ammonium sulfate suspension.

Introduction

Fluorochrome-labeled reagents are useful in immunological assays and procedures. Fluorescence detection is advantageous due to its high resolution along with the capabilities of being able to stain live cells and perform double-labeling techniques and cell sorting procedures.

Fluorescence is a phenomenon that results from a fluorochrome absorbing incident light of a particular wavelength, thus becoming "excited" so that the fluorochrome's electrons are boosted to a higher energy level. After these electrons return to their original energy state, energy is "emitted" as light of a characteristic wavelength. An epifluorescent microscope is used to generate and detect fluorescence. The light energy for excitation is transmitted through the objective lens onto the surface of the specimen. Filters are used to selectively excite the specimen with specific wavelengths of light. A second set of filters in the viewing light path blocks wavelengths of light such that the image transmitted is formed only by the emitted light. A black background and a high-resolution image is formed by such an arrangement.

Several different fluorescent compounds are available for use with immunological assays and procedures. Each different fluorophore will possess a distinct and characteristic excitation and emission spectra. Since different fluorochromes can be selected that have non-overlapping emission spectra, two fluorochromes can be visualized on the same sample. Thus, double-labeling studies can be carried out on two different antigens even if they possess identical subcellular distributions. (See Table 1), illustrates the wavelengths for excitation and emission for several fluorophores.

Phycobiliproteins

Phycobiliproteins are proteins found in red and blue-green algae. These proteins are nearly non-fluorescent in intact cells, but in solution they are very fluorescent in the purified state. This class of proteins possesses several characteristics that make them well suited for use in fluorescence-based immunological techniques such as cell sorting and immunohistochemical staining. These characteristics include:2,3

1. Their ability to excite and monitor emission at the red end of the spectrum, thereby decreasing the potential for interferences from biological matrices. They exhibit a large Stokes shift, thereby decreasing potential interferences from Rayleigh and Raman scatter along with other fluorescing substances.
2. Their fluorescence is not quenched by naturally occurring biological components.
3. The proteins are very soluble in aqueous environments and thereby exhibit minimal nonspecific binding.
4. They possess high absorbance coefficients (up to 2.4 X 10^6 M^-1 cm^-1) due to high bilin-chromophore content.
5. They exhibit a high fluorescence quantum yield (up to Q = 0.9) that is essentially independent of temperature and pH over a broad range.
The spatial arrangement of the bilins within the protein negates concentration quenching and imparts the steric protection that enables the fluorescence to be independent of pH.²,⁴

**Pierce’s R-Phycerythrin and Allophycocyanin**

R-Phycerythrin is a red fluorescent, multi-subunit protein that can be isolated from the marine algae *Porphyra tenera* or *Gastroclonium coulterii*. This fluorochrome is a member of the phycobiliprotein family of proteins that can be found in blue-green algae, red algae, and cryptomonads. In these organisms, phycobiliproteins play critical roles in transferring the energy absorbed from light to chlorophyll where it can be utilized within the photosynthetic process. R-Phycerythrin has a molecular weight of 240,000 and consists of multiple subunits with an arrangement of (aβ)6; 34 bilins are contained in this protein to give an extremely high absorbance coefficient over a wide spectrum.² ∊₅₆₅ = 1.96 x 10⁶ M⁻¹ cm⁻¹.² The quantum yield of R-phycoerythrin at 578 nm is 0.82.⁵

Allophycocyanin is a red fluorescent, protein that can be isolated from *Gastroclonium coulterii* or *Anabaena variabilis*.⁴ Allophycocyanin has a molecular weight of 104,000 and consists of multiple subunits with an arrangement of (aβ)³. Two six bilins are contained in this protein to give a high absorbance coefficient (∊₆₅₀ = 7 x 10⁵ M⁻¹ cm⁻¹).² The quantum yield of allophycocyanin at 660 nm is 0.68.⁵,⁶

Pierce provides two different phycobiliproteins, R-phycoerythrin and allophycocyanin, which can be used to make conjugates with distinct advantages over other fluorescent conjugates.⁵ R-Phycerythrin and allophycocyanin conjugates also extend the capabilities of fluorescent analysis to include three- and four-color fluorescence activated cell sorting.⁻¹⁻¹⁰ For four-color analysis, fluorescein can be isolated at 488 nm with an argon ion laser and detected at 525 nm; R-phycoerythrin is excited with the argon ion laser at 488 nm and detected at 570 nm; a dye laser can excite Texas Red at 605 nm and it is detected at 620 nm; allophycocyanin is excited with the dye laser at 605 nm and detected at 645 nm.² The excitation and emission spectrum of R-phycoerythrin and allophycocyanin is shown in Figure 1.

**Example Protocol for Preparing Pyridyldisulfide Activated Phycobiliproteins**

The generic protocol shown below can be used to prepare pyridyldisulfide-activated phycobiliproteins. This protocol may require optimization for a particular application. A variety of crosslinkers can be used to prepare phycobiliprotein conjugates.⁴ The method below makes use of SPDP (Product No. 21857), LC-SPDP (Product No. 21651), or Sulfo-LC-SPDP (Product No. 21650). These crosslinkers react with primary amines on the surface of the phycobiliprotein and introduce pyridylsulfide group(s). The pyridylsulfide group can then be reacted with another protein that contains a free sulfhydryl, or it can be deprotected with a reducing agent to generate a free sulfhydryl group and this can then be reacted with a second maleimide-activated protein. Sulfo-SMCC (Product No. 22422) is a good choice for preparing maleimide-activated proteins.

**Materials**

A. SPDP, LC-SPDP or Sulfo-LC-SPDP stock solution: 20 mM SPDP or LC-SPDP in DMSO or 10 mM Sulfo-LC-SPDP in water.

   **Note:** Prepare just before use, since these reagents are prone to hydrolysis. (Since Sulfo-LC-SPDP is a water-soluble crosslinker it can be added directly to the phycobiliprotein solution to decrease the extent of hydrolysis.)

B. Phosphate buffered saline (PBS)/EDTA: 20 mM sodium phosphate, 150 mM NaCl, 1 mM EDTA, 0.02% sodium azide, pH 7.2.

C. Borate buffered saline (BBS): 50 mM sodium borate, 300 mM NaCl, pH 8.5.

D. Phycobiliprotein solution: Dialyze R-phycoerythrin or allophycocyanin into BBS and adjust protein concentration to 1 mg/ml.

   **Note:** These proteins are supplied in an ammonium sulfate suspension; extensive dialysis is necessary to remove extraneous ammonium ion which will quench the crosslinking reaction.

E. Desalting columns: 1 x 5 ml desalting column, for example Pierce’s crosslinked dextran desalting column (Product No. 43230).

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Fax 815-968-7163 or 800-842-5007
Method

1. To 1 mg of phycobiliprotein in 1.0 ml BBS, add 25 µl of the 20 mM stock solution of the SPDP or LC-SPDP.

   Note: 0.25-0.30 mg of Sulfo-LC-SPDP can be added directly to the phycobiliprotein solution, since this crosslinker is water-soluble.

2. React for 30 minutes at room temperature.

3. To remove the unreacted and hydrolyzed crosslinker, apply the sample to a 5 ml desalting column equilibrated with PBS. Elute with PBS and collect 1 ml fractions. The first peak to elute from the column (monitored at 280 nm) will be the pyridyldisulfide-activated phycobiliprotein.

   The pyridyldisulfide-activated phycobiliprotein can now be reacted with another protein that contains a free sulfhydryl following the protocol for the specific cross-linker used. Alternatively, it can be deprotected with a reducing agent (e.g. 50-100 mM DTT) to generate a free sulfhydryl group and this can then be reacted with a second, maleimide-activated protein. Sulfo-SMCC (Product No. 22322) is a good choice for preparing maleimide-activated proteins.

References


Lissamine is a trademark of ICI Americas
Texas Red is a trademark of Molecular Probes, Inc.
Tween is a trademark of ICI Americas.

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Table 1: Excitation and emission wavelengths for various fluorophores.1

<table>
<thead>
<tr>
<th>Fluorophore</th>
<th>Excitation</th>
<th>Emission</th>
<th>Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMCA</td>
<td>345-350</td>
<td>440-460</td>
<td>Blue</td>
</tr>
<tr>
<td>(7-amino-4-methylcoumarin-3-acetic acid)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DAPI*</td>
<td>365</td>
<td>&gt;420</td>
<td>Blue</td>
</tr>
<tr>
<td>(4', 6-diamidino-2-phenylindole)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fluorescein</td>
<td>490-495</td>
<td>520-525</td>
<td>Green</td>
</tr>
<tr>
<td>Hoechst 33258*</td>
<td>360</td>
<td>470</td>
<td>Blue</td>
</tr>
<tr>
<td>Lissamine</td>
<td>570</td>
<td>590</td>
<td>Red</td>
</tr>
<tr>
<td>R-phycocyanin</td>
<td>555, 618-620</td>
<td>634-655</td>
<td>Red</td>
</tr>
<tr>
<td>B-phycoerythrin</td>
<td>545, 565</td>
<td>575</td>
<td>Red to Orange</td>
</tr>
<tr>
<td>R-phycoerythrin</td>
<td>480, 545, 565</td>
<td>578</td>
<td>Red to Orange</td>
</tr>
<tr>
<td>Allophycocyanin</td>
<td>650</td>
<td>660</td>
<td>Red</td>
</tr>
<tr>
<td>Rhodamine</td>
<td>546-552</td>
<td>570-580</td>
<td>Red</td>
</tr>
<tr>
<td>TRITC</td>
<td>515-520, 550-555</td>
<td>570</td>
<td>Red</td>
</tr>
<tr>
<td>(tetramethyl rhodamine isothiocyanate)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Texas Red</td>
<td>596</td>
<td>620</td>
<td>Red</td>
</tr>
<tr>
<td>Cy3 dye</td>
<td>553-555</td>
<td>568-574</td>
<td>Red</td>
</tr>
</tbody>
</table>

* DAPI and Hoechst 33258 are DNA intercalating agents and are useful in limiting the irradiation of other fluorochromes during focusing. Nuclei can be stained by these agents by washing the specimen with a 0.0005% solution.

Figure 1.

The excitation and emission spectrum of R-Phycoerythrin (R-PE) and allophycocyanin (APC).