## Phycobiliproteins: R-PE, B-PE, CPC, APC

### Product Information

<table>
<thead>
<tr>
<th>Product name</th>
<th>cat. number</th>
<th>MW (g·mol⁻¹)</th>
<th>( \lambda_{exc} ), ( \lambda_{em, max.} ) (nm)</th>
<th>mol. abs. (M⁻¹ cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>R-PhycoErythrin (R-PE)</strong></td>
<td>FP-28310A, 1mg</td>
<td>240 000</td>
<td>( \lambda_{exc} ): 498.546; ( \lambda_{em, max.} ): 566 nm / 576 nm</td>
<td>EC: 1.53x10⁶</td>
</tr>
<tr>
<td></td>
<td>FP-28310B, 5mg</td>
<td></td>
<td>QY: 0.84</td>
<td></td>
</tr>
<tr>
<td>Supplied in 100 mM K Phosphate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>buffer, pH7.0 with 60% saturated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(NH₄)₂SO₄, 1 mM EDTA and 1 mM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium Azide.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>B-PhycoErythrin (B-PE)</strong></td>
<td>FP-17885A, 1mg</td>
<td>240 000</td>
<td>( \lambda_{exc} ): 546.566 nm / 576 nm</td>
<td>EC(545nm) : 2.4x10⁶</td>
</tr>
<tr>
<td></td>
<td>FP-17885B, 5mg</td>
<td></td>
<td>QY: 0.98</td>
<td>EC(563nm) : 2.33x10⁶</td>
</tr>
<tr>
<td>Supplied Lyophilized</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>C-PhycoCyanine (C-PC)</strong></td>
<td>FP-35191A, 1mg</td>
<td>232 000</td>
<td>( \lambda_{exc} ): 620nm/642nm</td>
<td>EC: 1.54x10⁶</td>
</tr>
<tr>
<td></td>
<td>FP-35191B, 5mg</td>
<td></td>
<td>QY: 0.81</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**FP-17885A, 1mg**

Supplied Lyophilized
### Product name, cat.number, MW, \( \lambda_{\text{exc}} \), \( \lambda_{\text{em}} \) max., mol. abs. mol. \( \text{cm}^{-1} \), EC, QY:

<table>
<thead>
<tr>
<th>Product name, cat.number</th>
<th>MW (g·mol(^{-1}))</th>
<th>( \lambda_{\text{exc}} \lambda_{\text{em}} ) max. (nm)</th>
<th>mol. abs. (M(^{-1})cm(^{-1}))</th>
<th>EC</th>
<th>QY:</th>
</tr>
</thead>
<tbody>
<tr>
<td>AlloPhycocyanine, cross-linked (cl-APC)</td>
<td>105 000</td>
<td>651nm/662nm/QY: 0.68</td>
<td>105 000</td>
<td>651nm/662nm/QY: 0.68</td>
<td>EC: 7.3 ( \times 10^7 )</td>
</tr>
</tbody>
</table>

Supplied in saturated (NH\(_4\))\(_2\)SO\(_4\) soln.

---

**Storage:** +4°C (in the dark, avoid moisture, DO NOT FREEZE)

---

**Technical and scientific information**

**Applications**

As a result from their efficient features, phycobiliproteins allow higher detection sensitivity, and can be used in various fluorescence based techniques (Fluorimetry in microplate, Flow Cytometry, FISH, two or multicolor detections...).

Both R-PE and B-PE suit classic applications with Kr/Ar and Ar/He (best) lasers, while APC has higher wavelength applications.

PE is appreciated because of its intense fluorescence, and its suitability to be excited as fluoresceins. PE is widely used in fluorescence microarrays and microplate assays. B-PE has a stronger emission than R-PE. However R-PE has a 'spike' in excitation spectrum at around 488nm, that makes it better for use with the laser of most FCM and microarray readers. It is widely used in tandem with FITC, and even in triple or quadruple detection with FITC/[R-PE]/APC/PerCP (FCM).

However several reasons limit PE use, especially in microscopy:

- Significant spectral overlap between phycoerythrin and FITC needs to be corrected (compensation works well in FCM).
- Phycoerythrin big size (240Kda) prevents cell internal detections, or requires permeabilization procedures (as possible!).
- Phycoerythrin is prone to self-quenching, notably with high density antigens.

An alternative approach is to adopt a synthetic (small) fluorophore with an adequate excitation/emission/stocke’s shift, i.e. FluoProbes 494XXL or 480XXL.

PE, especially R-PE, has also been used to detect and measure antioxidants as peroxyl radicals.
C-PC accepts quanta from phycoerythrin by fluorescent energy transfer. Also, its red fluorescence can be transferred to allophycocyanin.

APC is ideal for He/Ne laser, double labeling with Sulfo-Rhodamine 101 or any other equivalent fluorochrome. Its near-infrared fluorescence is relatively free of interference from the auto fluorescence of cellular components and other biological materials. It is > 10 times more sensitive than conventional organic fluorophores and has been used in applications such as flow cytometry, homogeneous FRET assay, immunofluorescent staining and other immunoassays. It is provided as a cross-linked product to stabilize the most fluorescent form (trimmer).

APC XL has stabilized subunits by cross-linker. The spectral properties are the same has the original APC. But the natural APC is unstable at very low dilution. APC XL is recommended for stringent applications.

Reconstitution for lyophilized products
Reconstitute whole bottle with PBS or other adequate buffer (i.e. conjugation buffer) to adjust the concentration for further use. Mix well to get homogeneous solution and ensure no visible precipitate. Avoid foaming.

Note: It is not accurate to measure these phycobiliproteins directly by weight. To get an accurate weight we recommend to use its extinction coefficient

\[ [R-PE] = 0.122 \times A_{565} \]
\[ [B-PE] = 0.1 \times A_{545}, \]
\[ [APC] = 0.149 \times A_{651} \]

where \([\ ]\) is the concentration in mg/ml and \(A\) (i.e. \(A_{565nm}\)) is the absorbance (at 565nm).

Applications
• Phycobiliproteins (PE, PC and APC) are widely used for fluorescent labeling of antibodies and other probes. See below guide lines for conjugation. Labeled probes can be used in a variety of techniques
• Phycobiliproteins, especially R-PE, are also used to detect and measure antioxidants as peroxylys radicals. Please refer to the literature.

Usage, Conjugation
Reconstituted RPE or APC solution The ammonium sulfate has to be removed by dialyzed or desalting column.

Mix thoroughly PE until no precipitate is observed to get homogeneous solution. Further removal all the remaining ammonium sulfate and buffer exchange to the conjugation buffer.

For dialysis, use your conjugation buffer or PBS with 2 changes of buffer.

Conjugation protocols can found in the literature and in the technical sheets of our crosslinking agents, such as SMCC #UP17412A, MAL-PEOx-NHS or BS3 #UP24864A.

R-PhycoErythrin (R-PE) FP-28310B, 5mg
R-PE is a phycobiliprotein purified from red algae. It is made up of (α)6βϒ subunit structure.

SPECIFICATIONS: Purity: A566/A280≥5.5, A566/A498≤1.5
Impurity A620/A566≤0.005

PROPERTIES: MW: 240kDa
Absorption Max: 566nm | Emission Max: 575nm
Extinction Coefficient: $1.96 \times 10^6$ cm$^{-1}$ M$^{-1}$ | Quantum Yield: 0.84 |

PACKAGING: R-PE is supplied in 100 mM Potassium Phosphate buffer, pH 7.0 with 60% Saturated $(\text{NH}_4)_2\text{SO}_4$, 1 mM EDTA and 1 mM Sodium Azide.

STORAGE Store R-PE in dark at 2–8 °C. Do not freeze.

USE INFORMATION:
Mix thoroughly R-PE until no precipitate is observed to get homogeneous solution.
Remove all the remaining ammonium sulfate and exchange buffer to the conjugation buffer.

SMCC activated R-PhycoErythrin (R-PE)
SMCC activated R-PhycoErythrin allows instantaneous conjugation of R-PE with SH bearing molecules or 2-IT activated target, maintaining 1:1 ratio.

SPECIFICATIONS:
- Purity: $A_{565}/A_{280} \geq 5.3$
- Coupling ratio SMCC/RPE: 1.5–3

PACKAGING:
- R-PE is supplied in 10 mM Potassium Phosphate buffer with sugar as additive. No ammonium sulfate or other material may interfere conjugation.

STORAGE
Store in dark at 2–8°C with desiccant. Do not freeze.

TECHNICAL INFO
SMCC-RPE is treated with SMCC to get only 1.5–3 SMCC per RPE ensuring the best yield of conjugate with minimal aggregates. It has been purified to remove excess SMCC lyophilized, and lyophilized, thus quite suitable for long term storage without losing the maleimide reactivity and keep high quality of RPE with perfect conjugation consistency.

USAGE:
Notes
- **Weight**: One bottle of Lyo SMCC-RPE contains 2 mg of RPE with sugar as additive. It is not accurate to measure activated RPE directly by weight.
- **Buffer**: The product contains no ammonium sulfate or other material that may interfere conjugation is contained in this product. Buffer such as phosphate or carbonate/bicarbonate are most often used. It is recommended to add 2-5 mM EDTA to prevent the oxidation of sulfhydryl groups (i.e., formation of disulfide bonds). Buffer containing primary amines, reducing agents or sodium azide should be avoided.
- **Stability**: No preservative is added in the product. Once reconstitute it, use it as soon as possible (it is not suitable to store after reconstitution).

Reconstitution
Reconstitute whole bottle of lyophilized SMCC-RPE (2 mg) with your conjugate buffer to adjust the concentration for further use.

Procedure for Coupling SMCC-RPE with Thiol Group
Reaction scheme of SMCC-RPE react with thiol group containing protein. The maleimides on SMCC-RPE will react with sulfhydryl group on protein under mild conditions of temperature and pH to form RPE-protein conjugate.
AlloPhycocyanin, Crosslinked (cl.APC)  FP-35298A, 1mg

AlloPhycocyanin (APC) is a fluorescent phycobiliprotein purified from red algae. Crosslinked AlloPhycocyanin (cl.APC) is chemically cross-linked without changing APC’s fluorescent property, designed for labeling applications.

PROPERTIES:

MW: 105kDa
Absorption Max: 650nm | Emission Max: 662nm
Extinction Coefficient: 7.0 × 10^5 cm⁻¹ M⁻¹ | Quantum Yield: 0.68

SPECIFICATIONS:

Purity: 650/A280 ≥ 5.0, A650/A620≥1.5
Impurity A620/A566≤0.005
Cross Link Ratio ≥ 1.2

PACKAGING: R-PE is supplied in 100 mM Potassium Phosphate buffer, pH7.0 with 60% Saturated (NH₄)₂SO₄, 1 mM EDTA and 1 mM Sodium Azide.

Store R-PE in dark at 2-8 °C. Do not freeze.

USE INFORMATION:

Mix thoroughly R-PE (5mg) in (5ml) ultrapure water until no precipitate is observed to get homogeneous solution (1mg/ml).

Exchange buffer by the desired conjugation buffer, using suitable means (dialysis) to remove contained ammonium sulfate.

Streptavidin lyophilized powder  51558K, 10 mg
recombinant protein in E.coli

PROPERTIES:

Lyophilized powder, essential salt free
Molecular Weight :~53 kDa (based on Mass) ~56 KDa (based on Page)
Excitation Coefficient :E_280nm @ 1% = 32; E_280nm @1 mg/ml =3.2

SPECIFICATIONS:

Activity : ≥ 17 (HABA)
Solubility : ≥ 10mg/ml (in MiliQ water)
≥ 50mg/ml (in neutral or alkaline buffer)

SDS Page: one major band

STORAGE

Store at 4°C with desiccant.

USAGE:

Applications

• Immunoassays
• Histochemistry, techniques
• Flow cytometry, Microarrays, Blot analysis,…
• FISH (Fluorescence In Situ Hybridization), DNA hybridization
• Immunopurification:
• Isolate biotinylated molecules
• Others: i.e. MHC tetramer technology

Reconstitution
FT-28310A

Centrifuge vial prior to opening. Reconstitute whole bottle of Streptavidin (10 mg) with your buffer to adjust the concentration for further use. The core Streptavidin has a near neutral pI, therefore reconstitution by neutral buffer may cause precipitation and should be followed by centrifugation. Frozen aliquots are stable for at least 6 months when stored at −20°C. Avoid repeated freeze-thaw cycles.

Legals
For research or further manufacturing use only.
Reconstitution Reconstitute whole bottle of Lyo SMCC-RPE (2 mg) with your conjugate

Related products

- R-PE labeling kit-NH2 #BT3821
- R-PE labeling kit-SH #BT3831
- B-PE labeling kit-NH2 #BT3801
- B-PE labeling kit-SH #BT3811
- APC labeling kit-NH2 #BT3841
- APC labeling kit-SH #BT3851
- Accessory reagents:
  - Accessory reagents:
  - PBS Buffer tabs #UP307157, packs #UP68723A
  - SMCC #UP17412A
  - MAL-PEO-NHS #AL6580
  - Iminothiolane #42425A, CF617A (Traut’s reagent)
  - SATA #UP84235A (N-Succinimidyl S-acetylthioacetate)

Ordering information

Catalog size quantities and prices may be found at http://www.interchim.com
Please inquire for higher quantities (availability, shipment conditions).

For any information, please ask: FluoProbes® / Interchim; Hotline: +33(0)4 70 03 73 06

Disclaimer: Materials from FluoProbes® are sold for research and manufacturing use only, and are not intended for food, drug, household, or cosmetic use. FluoProbes® is not liable for any damage resulting from handling or contact with this product.

Rev.R10E-G09E

P.6