

## Product Information

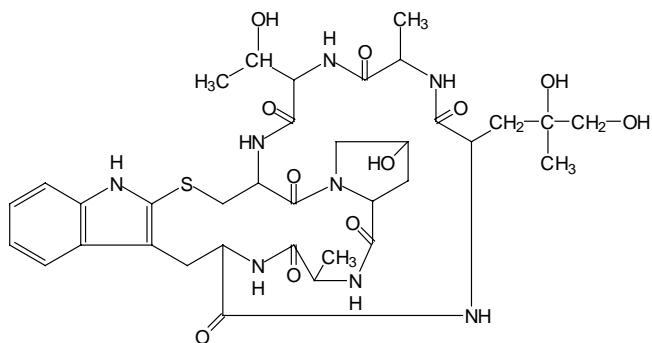
### PHALLOIDIN, AND PHALLOIDIN CONJUGATES

Phalloidin-CPTIC, Phalloidin-FITC, and Phalloidin-TRITC

#### PRODUCT NUMBERS:

Phalloidin P2141  
 Phalloidin -CPITC P8543  
 Phalloidin -FITC P5282  
 Phalloidin -TRITC P1951

#### PRODUCT DESCRIPTION



#### PHYSICAL PROPERTIES OF PHALLOIDIN P2141:

Appearance: White powder  
 Melting Point: 280-282 °C<sup>1</sup>  
 Molecular Formula: C<sub>35</sub>H<sub>48</sub>N<sub>8</sub>O<sub>11</sub>S  
 Molecular Weight: 788.9 (anhydrous)  
 Extinction Coefficient: E<sup>1%</sup> = 0.597 at 295 nm in water<sup>2</sup>  
 Storage Temperature: Room Temperature

#### PHYSICAL PROPERTIES OF PHALLOIDIN-CPTIC P8543:

Molecular Formula: C<sub>58</sub>H<sub>71</sub>N<sub>11</sub>O<sub>11</sub>S<sub>4</sub>  
 Molecular Weight: 1226.5  
 Excitation: 387 nm<sup>2</sup>  
 Emission: 465-470 nm<sup>2,3</sup>  
 Molar Extinction Coefficient: 15,000 (387 nm)<sup>3</sup>  
 Storage Temperature: -0°C

#### PHYSICAL PROPERTIES OF PHALLOIDIN-FITC P5282:

Molecular Formula: C<sub>58</sub>H<sub>63</sub>N<sub>10</sub>O<sub>14</sub>S<sub>4</sub>  
 Molecular Weight: 1252.4  
 Excitation: 495 nm<sup>3</sup>  
 Emission: 513 nm<sup>3</sup>  
 Molar Extinction Coefficient: 70,000 (495 nm)<sup>3</sup>  
 Storage Temperature: -0°C

#### PHYSICAL PROPERTIES OF PHALLOIDIN-TRITC P1951:

Molecular Formula: C<sub>62</sub>H<sub>72</sub>N<sub>10</sub>O<sub>12</sub>S<sub>4</sub>  
 Molecular Weight: 1305.6  
 Excitation: 540-545 nm<sup>3,4</sup>  
 Emission: 570-573 nm<sup>3,4</sup>  
 Molar Extinction Coefficient: 80,000 (545 nm)<sup>3</sup>  
 Storage Temperature: -0°C

Phalloidin is a fungal toxin isolated from the poisonous mushroom *Amanita phalloides*. Phalloidin's toxicity is attributed to its ability to bind to F actin in liver and muscle cells. As a result of binding phalloidin, actin filaments become strongly stabilized. Phalloidin has been found to bind only to polymeric and oligomeric forms of actin and not to monomeric actin. The dissociation constant of the actin-Phalloidin complex has been determined to be on the order of 3 X 10<sup>-8</sup> M.<sup>5</sup> Phalloidin differs from amanitin in rapidity of action; at high dose levels, death of mice or rats occurs within 1 or 2 hours.<sup>1</sup>

Fluorescent conjugates of phalloidin are used to label actin filaments for histological applications.<sup>2,3,4,5,6,7,8</sup>

Some structural features of phalloidin are required for its binding to actin. However, the side chain of amino acid 7 (γ-δ-dihydroxyleucine) is accessible for chemical modifications without appreciable loss of affinity for actin. CPITC-phalloidin<sup>2,3</sup>, FITC-phalloidin<sup>3,5</sup>, and TRITC-phalloidin<sup>3,6</sup> conjugates are offered by Sigma for these applications. The TRITC conjugate is considered less susceptible to photobleaching than the FITC conjugate.<sup>6</sup>

#### Precautions and Disclaimer

**WARNING:** Extremely hazardous! Be aware of the risks and familiar with safety procedures before you use this product.

#### Toxicity Data for P2141:

Intraperitoneal Injection LD50: 2 mg/kg in mice<sup>9</sup>

#### Solubility/Solution Stability

Solubility in water (0°C): 0.5%; much more soluble in hot water; freely soluble in methanol, ethanol, butanol, and pyridine.<sup>1</sup>

Sigma tests the solubility of these products in methanol at the following concentrations:

Phalloidin P2141: 10 mg/ml  
Phalloidin -CPITC P8543: 1 mg/ml  
Phalloidin -FITC P5282: 0.5 mg/ml  
Phalloidin -TRITC P1951: 1 mg/ml

Solutions should be prepared fresh and protected from light when ever possible.

#### **Procedure**

Stock solutions of Phalloidin conjugates have been made in methanol or DMSO at 0.1 to 5 mg/ml. Final dilutions made in aqueous physiological buffers for staining range from 0.1  $\mu$ M to 100  $\mu$ M with corresponding incubation times of 15 minutes to 72 hours. A typical application for staining cells follows.<sup>5</sup>

- 1) Cells are washed with phosphate buffered saline (PBS)
- 2) Cells are fixed for 5 minutes in 3.7 % formaldehyde in PBS. Then washed extensively in PBS.
- 3) Cells may be dehydrated with acetone, permeabilized with 0.1% Triton X-100 in PBS, and washed again in PBS.

- 4) Cells are stained with a 50 ug/ml fluorescent phalloidin conjugate solution in PBS (containing 1% DMSO from the original stock solution) for 40 minutes at room temperature.
- 5) Wash several times with PBS to remove unbound phalloidin conjugate.

#### **References**

1. The Merck Index, 12<sup>th</sup> ed., p. 1238 # 7336 (1996).
2. Small, J. V. et al., *J. Cell Science*, 89, 21 (1988).
3. Faulstich, H. et al., *J. Muscle Res. Cell Motility*, 9, 370 (1988).
4. Waggoner, A. et al., *Methods in Cell Biology*, 30, 449 (1989).
5. Wulf, E. et al., *Proc. Nat. Acad. of Sci., USA*, 76, 4498 (1979).
6. Faulstich, H. et al., *Exp. Cell Res.*, 144, 73 (1983).
7. Lawson, M.A., and Maxfield, F.R., *Nature*, 377, 75 (1995).
8. Heidecker, M. et al., *Biochemistry*, 34, 11017 (1995).
9. *New Eng. J. Med.*, 269, 223 (1963).

rbg 11/03/00