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### **Product Information**

# Phalloidin from *Amanita phalloides* and Phalloidin Conjugates (Coumarin, FITC, and TRITC)

Phalloidin, Catalog Number **P2141**Phalloidin, Coumarin labeled, Catalog Number **P2495**Phalloidin, Fluorescein isothiocyanate labeled,
Catalog Number **P5282**Phalloidin, Tetramethylrhodamine B isothiocyanate,
Catalog Number **P1951** 

### **Product Description**

### Physical Properties Of Phalloidin

(Catalog Number P2141):
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: 2 F1% = 0.597 (2)

Extinction Coefficient:<sup>2</sup> E<sup>1%</sup> = 0.597 (295 nm in water)

Store at Room Temperature

### Physical Properties Of Phalloidin, Coumarin labeled

(Catalog Number P2495): Molecular Formula: C<sub>62</sub>H<sub>75</sub>N<sub>11</sub>O<sub>15</sub>S<sub>2</sub> Molecular Weight: 1278.45 Excitation:<sup>2</sup> 384 nm

Emission:<sup>2,3</sup> 470 nm

Molar Extinction Coefficient:4 10,000 (275 nm in

ethanol)

Storage Temperature -20 °C

## Physical Properties Of Phalloidin, Fluorescein isothiocyanate labeled

(Catalog Number 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:<sup>3</sup> 495 nm

Emission:<sup>3</sup> 513 nm

Molar Extinction Coefficient:<sup>3</sup> 70,000 (495 nm)

Storage Temperature –20 °C

# <u>Physical Properties Of Phalloidin, Tetramethyl-rhodamine B isothiocyanate (TRITC)</u>

(Catalog Number P1951): Molecular Formula: C<sub>60</sub>H<sub>70</sub>N<sub>12</sub>O<sub>13</sub>S<sub>2</sub> Molecular Weight: 1231.41

Excitation:<sup>3,5</sup> 540–545 nm Emission:<sup>3,5</sup> 570–573 nm

Molar Extinction Coefficient:<sup>3</sup> 80,000 (545 nm)

Storage Temperature -20 °C

Phalloidin is a fungal toxin isolated from the poisonous mushroom *Amanita phalloides*. Its toxicity is attributed to the ability to bind 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  $3 \times 10^{-8}$  M.<sup>6</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.  $^{2,3,5-9}$  Some structural features of phalloidin are required for the binding to actin. However, the side chain of amino acid 7 ( $\gamma$ - $\delta$ -dihydroxyleucine) is accessible for chemical modifications without appreciable loss of affinity for actin. Coumarin, FITC,  $^{3,6}$  and TRITC  $^{3,7}$  phalloidin conjugates are offered for these applications. The TRITC conjugate is considered less susceptible to photobleaching than the FITC conjugate.  $^7$ 

### **Precautions and Disclaimer**

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

### **Preparation Instructions**

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

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: 10 mg/ml

Phalloidin-Coumarin: 1 mg/ml Phalloidin-FITC: 0.5 mg/ml Phalloidin-TRITC: 0.5 mg/ml

### **Procedure**

Stock solutions of phalloidin conjugates have been made in methanol or DMSO at 0.1–5 mg/ml. Final staining solutions in aqueous physiological buffers are in the concentration range of 0.1–100  $\mu$ M with corresponding incubation times of 15 minutes to 72 hours. The following procedure may be used as a guideline for staining cells:5

- Cells are washed with phosphate buffered saline (PBS).
- Cells are fixed for 5 minutes in 3.7% formaldehyde solution 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  $\mu$ g/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

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