Certificate of Analysis

Flamma™ P Series

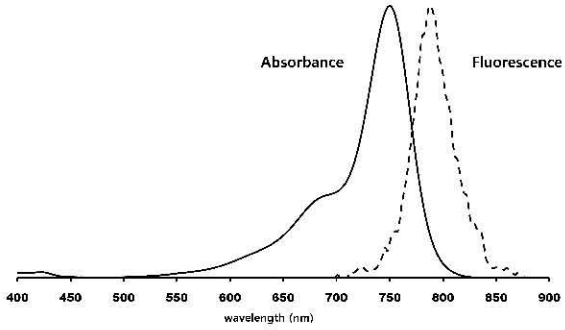
FPI-749

1. Product Information

Chemical Structure

Available Upon Request

UV/PL Spectrum



Code No.	PWA1308
Lot. No.	
Formula	$C_{41}H_{53}N_3O_9S_3$
Molecular Weight	828.07
Absorption Wavelength (Maximum Peak, Room Temperature)	750 nm (1xPBS buffer, 3.02 x 10^{-6} M) 750 nm (Distilled water, 3.02 x 10^{-6} M) 753 nm (Methanol, 3.02 x 10^{-6} M)
Molar Extinction Coefficient (Maximum Peak, Room Temperature)	2.22 × 10^5 $M^{-1}cm^{-1}$ (1xPBS buffer, 3.02 x 10^{-6} M) 2.29 × 10^5 $M^{-1}cm^{-1}$ (Distilled water, 3.02 x 10^{-6} M) 3.03 × 10^5 $M^{-1}cm^{-1}$ (Methanol, 3.02 x 10^{-6} M)
Emission wavelength (Maximum Peak, Room Temperature)	782 nm (1xPBS buffer, 3.02 x 10^{-6} M, ex. at 750 nm) 783 nm (Distilled water, 3.02 x 10^{-6} M, ex. at 750 nm) 792 nm (Methanol, 3.02 x 10^{-6} M, ex. at 753 nm)
Usable pH range	5 ~ 12 (8.5 ~ 9.5 recommended labeling pH)
Usable Temperature	~ 60 ℃ (25 ~ 37 ℃ recommended labeling temperature)
Usable Solvent for Stock Solution	water, DMF, DMSO (water recommended solvent)
Purity	99%
Storage Conditions (recommended)	Store refrigerated at 2 ~ 8 ℃ in the dark. (before use) Store refrigerated at -20 ℃ in the dark. (stock solution)
Date of Manufacture	
Date of Expiration	

2. Product Feature

- This product can be dissolved in distilled water, DMF and DMSO for preparing stock solution.
- Stock solutions can be diluted in water, alcohol and various buffers that are used in chemical and biological experiments such as PBS buffer.
- Conjugation with protein, peptide, antibody, biopolymer, organic compound, and nanoparticle including amino group is possible.
- Long-term storage is possible at room temperature when dissolved in water, DMF or DMSO, but it is recommended to store at -20 ℃.

3. Labeling Protocol for Protein

- Dye solution
  ① Dissolve dye (1 mg Flamma™ dye / 50 μl DMF or DMSO).
  ② Dilute the dye solution (+50 μl distilled water).
  ③ Store in refrigerated at -20 ℃.

- Example for albumin protein (from bovine serum)
  ① Prepare protein solution with 0.1M phosphate (or carbonate) buffer (pH 9) (1 mg protein / 0.1 ml buffer).
  ② Mix dye solution 1 μl and protein solution 19 μl.
  ③ Incubate more than 1 hour at room temperature.
  ④ Filter the labeled protein using the membrane filter.

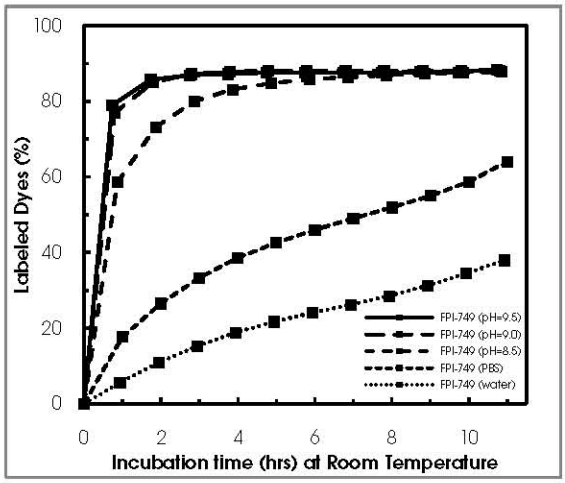
※ This procedure has been optimized for albumin (from bovine serum) protein. However, most of water soluble proteins are also labeled by this procedure.
※ You can select a proper filter or a suitable gel separation column for the purpose of separating the excess dyes which did not label.
※ If you want to label protein/antibody product in its stable buffer or unstable protein/antibody in basic condition, we recommend the next protocol as follow.
  ① Mix dye solution 1 μl and protein solution (its stable buffer or DMF or DMSO) 19 μl.
  ② Add 4 μl (2 ~ 6 μl) 0.1M phosphate (or Carbonate) buffer (pH 9) to the reaction solution
  ③ Mix vigorously and incubate 1 hour at room temperature.
  ④ Filter the labeled protein using the membrane filter.

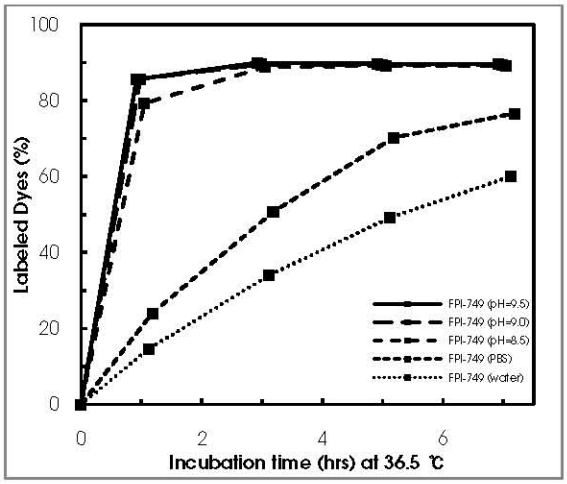
- Protocol for Peptide
  ① Dissolve 1 mol peptide. (1 mg peptide / 0.1 ml DMF or DMSO)
  ② Mix 1 mol dye solution (or dye) and vortex. (Total Volume X ml)
  ③ Double the total volume with 0.1M phosphate (or Carbonate) buffer (pH 9).
  ④ Incubate for 4 hours at room temperature.
  ⑤ Separate the labeled peptide through HPLC.

※ When your peptide doesn't dissolve perfectly in DMF (or DMSO), it is exchanged the dissolving solvent such as methanol, ethanol and dioxane.
※ If your peptide is unstable in basic condition, it is reacted in neutral buffer (pH 7.5 0.1M phosphate (or Carbonate) buffer) for more than 12 hrs.
※ In case your peptide is water-soluble and stable in basic condition, it can be labeled in pH 9.5 0.1M phosphate (or Carbonate) buffer) in 1 hour.

4. Labeling Information (Flamma™ P series) for Researchers

- Reaction temperature range : 4 ~ 60 ℃ (the best reaction rate - 37 ℃)
- Reaction pH range : pH 7.5 ~ 11 (the best reaction rate - pH 9.5)





- Reaction solvent : 0.1M carbonate or phosphate buffer
- Usable reaction solvent : DMF, DMSO, Alcohol (Methanol, Ethanol), distilled water, neutral pH carbonate/phosphate/Tris buffer and most of neutral/basic biological buffer
- Reaction Time : 30 min ~ 24 hours (recommended more than 2 hours)
- The recommended usage of Flamma™ P series dye
  - ① Organic Compound/ Peptide : equivalent
  - ② Protein/Antibody : equivalent ~ 0.25 mg dye per 1 mg protein/antibody
  - ③ Polymer : 1 mg (or what you want) dye per 1 mg polymer

5. Safety Warnings and Precautions

① Warning: For research only. Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

② We recommend that this products and components are handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. As all chemicals should be considered as potentially hazardous, it is advisable when handling chemical reagents to wear suitable protective clothing, such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In case of contact with skin or eyes, wash immediately with water.

③ Caution: This dye is intensely colored and very reactive. Care should be exercised when handling the dye vial to avoid staining clothing, skin, and other items.

Michael Chung, QC Manager

June 1, 2010