

Product Information

Phosphatase Substrate

Capsules

Preweighed 40 mg Capsules, Catalog No. **P5744**

Preweighed 100 mg Capsules, Catalog No. **P5869**

Storage Temperature -20 °C

Tablets

5 mg Tablets, Catalog No. **S0942**

40 mg Tablets, Catalog No. **P5994**

Storage Temperature -20 °C

Powder

Powdered Substrate, Catalog No. **P4744**

Storage Temperature -20 °C

Product Description

Formula: $C_6H_4NO_6PNa_2 \cdot 6H_2O$

Formula Weight: 371.14

p-Nitrophenyl phosphate (pNPP) is a soluble substrate for use with alkaline phosphatase conjugates in ELISA procedures. It may also be used for the determination of alkaline and acid phosphatase activity in physiological fluids and other aqueous solutions. This substrate produces a soluble end product that is yellow in color and can be read spectrophotometrically at 405 nm. The pNPP reaction may be stopped with 3 M NaOH solution and read at 405 nm.

Preparation Instructions

Dissolve tablets or contents of capsules to the desired concentration in either of the following buffers:

0.1 M glycine, pH 10.4, with 1 mM $MgCl_2$ and 1 mM $ZnCl_2$,

or

1 M diethanolamine, pH 9.8, with 0.5 mM $MgCl_2$

Typically a pNPP concentration of 1 mg/ml is used.

To prepare 0.1 M glycine buffer, pH 10.4, with 1 mM $MgCl_2$ and 1 mM $ZnCl_2$:

Add 7.51 g of glycine (Catalog No. G7126), 203 mg of $MgCl_2$ (Catalog No. M0250), and 136 mg of $ZnCl_2$

(Catalog No.208086) to 980 ml of water and mix. Adjust the pH to 10.4 with 19 M NaOH solution and adjust the volume to 1 L with water.

To prepare 1 M diethanolamine buffer, pH 9.8, with 0.5 mM $MgCl_2$:

Add 97 ml of diethanolamine (Catalog No. D8885) and 100 mg of $MgCl_2$ (Catalog No. M0250) to 800 ml of water, adjust the pH to 9.8 with 10 M HCl solution and adjust the volume to 1 L with water.

The alkaline phosphatase reaction in a multiwell plate may be stopped by the addition of 50 μ l of 3 M NaOH solution per 200 μ l of reaction mixture.

Related Products

4-Nitrophenol is the hydrolysis product of p-nitrophenyl phosphate (pNPP) and may be used as a standard to determine enzyme activity. It has a formula ($C_6H_5NO_3$) weight of 139.11.

Standard solutions can be prepared from the powdered product (Catalog No.1048) in 0.02 to 1 M sodium hydroxide solution.

A 10 mM 4-nitrophenol solution (Catalog No. N7660) is also available.

RC,GY,PHC 01/14-1