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ProductInformation

Phosphatase Substrates

Capsules

Storage Temperature: –0 °C
Preweighed 40 mg Capsules, Prod. No. P5744
Preweighed 100 mg Capsules, Prod. No. P5869

Tablets

Storage Temperature: –0 °C
5 mg Tablets, Prod. No. S0942
40 mg Tablets, Prod. No. P5994

Powder

Storage Temperature: –0 °C
Powdered Substrate, Prod. No. P4744
$C_6H_4NO_6PNa_2 \bullet 6H_2O$
FW 371.1

p-Nitrophenol (Hydrolysis Product) Standards

p-Nitrophenol, Prod. No. N0286
Storage Temperature: RT

p-Nitrophenol 10 mM Solution, Prod. No. N7660
Storage Temperature: 2 to 8 °C
$C_6H_5NO_3$
FW 139.1

Product Description

p-Nitrophenyl Phosphate (pNPP) is a soluble substrate for use with alkaline phosphatase 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 3M NaOH and read at 405 nm.

Preparation Instructions

Dissolve tablets or contents of capsules in either 0.1 M glycine buffer containing 1 mM $MgCl_2$ and 1 mM $ZnCl_2$, pH 10.4 **or** 1M diethanolamine buffer containing 0.5 mM $MgCl_2$, pH 9.8, to the desired concentration (typically a pNPP concentration of 1 mg/ml is used).

To prepare 0.1 M glycine buffer, 1 mM $MgCl_2$, 1 mM $ZnCl_2$, pH 10.4: Add 7.51 g of glycine (Product No. G 7126), 203 mg $MgCl_2$ (Product No. M0250) and 136 mg $ZnCl_2$ (Product No. Z4875) to approximately 980 ml dH_2O and mix. Adjust the pH to 10.4 with 19 N NaOH and adjust the volume to 1 L with dH_2O.

To prepare 1 M diethanolamine buffer 0.5 mM $MgCl_2$, pH 9.8: Add 97 ml diethanolamine (Product No. D 8885), 100 mg $MgCl_2$ (Product No. M0250) and 0.2 g sodium azide (Product No. S2002) to 800 ml dH_2O, adjust the pH to 9.8 with 10 M HCl and adjust the volume to 1 L with dH_2O.

The reaction may be stopped by the addition of 50 µl of 3 N NaOH per 200 µl of reaction mixture.

p-Nitrophenol standard solutions can be prepared in 0.02 to 1 N sodium hydroxide.

RG 1/03