

## Product Information

### Phosphatase Substrate

Suitable for manufacturing of diagnostic kits and reagents

Catalog Number **SRE0026**

Storage Temperature  $-20\text{ }^{\circ}\text{C}$

Synonyms: pNPP disodium salt, hexahydrate;  
*p*-nitrophenyl phosphate disodium salt, hexahydrate;  
*p*-nitrophenylphosphate disodium salt, hexahydrate

### Product Description

Formula:  $\text{C}_6\text{H}_4\text{NO}_6\text{PNa}_2 \cdot 6\text{H}_2\text{O}$

Formula Weight: 371.14

*p*-Nitrophenyl phosphate (pNPP) is a substrate used with alkaline phosphatase conjugates in such applications as ELISA procedures. Hydrolysis of pNPP produces *p*-nitrophenol, a soluble end product. The pNPP reaction is stopped with the addition of 3 M NaOH solution and the intensity of the yellow color is determined spectrophotometrically at 405 nm.

In physiological samples and model studies, pNPP has been used to determine alkaline phosphatase<sup>1-3</sup> and acid phosphatase activity.<sup>4,5</sup> Other applications that use pNPP include studies of cytokine levels.<sup>6</sup>

### Precautions and Disclaimer

For further (non-TSCA only use in US) manufacturing uses only. Not intended for direct use in humans or animals. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

### Storage/Stability

This product has a recommended retest date of one year when properly stored at  $-20\text{ }^{\circ}\text{C}$ .

### Preparation Instructions

A pNPP solution with a concentration of 1 mg/mL is typically used. Initial stock solutions of pNPP can be prepared in either of two buffers:

- 0.1 M glycine, pH 10.4, with 1 mM  $\text{MgCl}_2$  and 1 mM  $\text{ZnCl}_2$   
or
- 1 M diethanolamine, pH 9.8, with 0.5 mM  $\text{MgCl}_2$

A. To prepare 0.1 M glycine buffer, pH 10.4, with 1 mM  $\text{MgCl}_2$  and 1 mM  $\text{ZnCl}_2$ :

1. Add 7.51 g of glycine, 203 mg of  $\text{MgCl}_2$ , and 136 mg of  $\text{ZnCl}_2$  to 980 mL of water and mix.
2. Adjust the pH to 10.4 with 19 M NaOH solution.
3. Adjust the volume to 1 L with water.

B. To prepare 1 M diethanolamine buffer, pH 9.8, with 0.5 mM  $\text{MgCl}_2$ :

1. Add 97 mL of diethanolamine and 100 mg of  $\text{MgCl}_2$  to 800 mL of water.
2. Adjust the pH to 9.8 with 10 M HCl solution.
3. Adjust the volume to 1 L with water.

The alkaline phosphatase reaction in a multiwell plate may be stopped by adding 50  $\mu\text{L}$  of 3 M NaOH solution per 200  $\mu\text{L}$  of reaction mixture.

**References**

1. Dulis, B., and Wilson, I.B., *Cancer Res.*, **38(8)**, 2519-2522 (1978).
2. Koo, H.C. *et al.*, *Clin. Vaccine Immunol.*, **11(6)**, 1070-1074 (2004).
3. Bel-Ochi, N.C. *et al.*, *J. Immunol. Methods*, **394(1-2)**, 107-114 (2013).
4. Foti, A.G. *et al.*, *Clin. Chem.*, **23(1)**, 95-99 (1977).
5. Laidler, P. *et al.*, *Biochem. Mol. Biol. Educ.*, **32(6)**, 400-409 (2004).
6. Böttcher, M.F. *et al.*, *Pediatr. Res.*, **47(1)**, 157-162 (2000).

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