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Product Information

4-Nitrophenyl phosphate disodium salt hexahydrate

5 mg of substrate tablets, Catalog Number **N9389**
15 mg of substrate tablets, Catalog Number **N2640**
20 mg of substrate tablets, Catalog Number **N2765**

Storage Temperature –20 °C

CAS RN 333338-18-4
Synonyms: pNPP, *p*-Nitrophenyl Phosphate, Phosphatase substrate

Product Description

4-Nitrophenyl Phosphate (pNPP) is the substrate of choice for use with alkaline phosphatase conjugates in Enzyme Linked Immunosorbant Assay (ELISA) procedures due to its high sensitivity.[1,2] ELISA applications utilizing pNPP may be read in timed assays or stopped with alkaline solutions for delayed readings.[3] 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 the addition of 3 M NaOH solution and read at 405 nm.

These products are supplied as 50 or 100 tablets per box, individually foil wrapped for ease of use, storage, and safety.

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

Tablets should be warmed to room temperature before use. Solutions should be freshly prepared.

Dissolve 1 tablet in either Glycine Buffer or Diethanolamine Buffer to the desired concentration (typically a pNPP concentration of 1 mg/ml is used).

Glycine Buffer (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 pH to 10.4 with 19 M NaOH and bring the volume to 1 L with water.

Diethanolamine Buffer (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 pH to 9.8 with 10 M HCl and bring the volume to 1 L with water.

Storage/Stability

The tablets should be stored –20 °C.

Procedure

For ELISA procedures with alkaline phosphatase conjugates, add 200 µl of substrate solution (typically 1 mg/ml) per well and incubate the plate in the dark for 30 minutes at room temperature. The absorbance can be read at 405 nm on a multiwell plate reader. The reaction may be stopped by the addition of 50 µl of 3 M NaOH per 200 µl of reaction mixture.

Troubleshooting

If the background is too high:

1. Use a blocking step prior to the application of the primary antibody. Normal Serum (5% v/v) from the same species as the host of the second antibody generally produces the best results.
2. Additional blocking agents for an ELISA are:
   a. 0.05% TWEEN® 20 in 50 mM TBS, pH 8.0.
   b. 1% BSA containing 0.05% TWEEN 20 in 50 mM TBS, pH 8.0.
   c. 3% nonfat-dried milk in 0.01 M TBS (Catalog No. P2194). Do not use milk as a blocking agent when using avidin-biotin systems.
3. Use 0.05% TWEEN 20 in all washing and antibody diluent buffers.
4. Run control wells without the primary antibody to check for non-specific reactivity of the secondary antibody/alkaline phosphatase conjugate.
5. Adjust the titer of the primary antibody and/or the alkaline phosphatase conjugate to determine the optimal working dilutions.

If no color develops or color is too faint:

1. Adjust the concentration of the primary antibody.
2. Adjust the concentration of the secondary antibody/alkaline phosphatase conjugate.
3. Determine if the enzyme conjugate is active by mixing a small sample of substrate and conjugate together in a test tube.
4. Increase the substrate incubation time or temperature.
5. Adjust the concentration of the coating antigen.
6. Consider using an amplifying system such as avidin-biotin.

References

1. Voller, A., et al., Enzyme immunoassays in diagnostic medicine. Theory and practice. Bull. World Health Organ., **53(1)**, 55-65 (1976).
2. Engvall, E., Enzyme immunoassay ELISA and EMIT. Methods Enzymol., **70(A)**, 419-439 (1980).
3. Voller, A., and Bidwell, D., in Manual of Clinical Laboratory Immunology, 3rd ed., Rose, N., et al., eds., American Society for Microbiology (Washington, D. C.: 1986), pp. 106-107.

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