Product Information

4-Nitrophenyl phosphate bis(cyclohexylammonium) salt

Phosphatase substrate

N3129

Product Description

CAS Registry Number: 52483-84-8

Synonyms: pNPP, *p*-Nitrophenyl Phosphate, *p*NPP bis(cyclohexylammonium) salt

Molecular Formula: $C_6H_4NO_6P \bullet (C_6H_{11}NH_3^+)_2$

Formula Weight: 417.44

λ_{max}: 311 nm[1]

Extinction Coefficient: E^{mM} = 9,867 (in 0.01 N NaOH)

Storage Temperature: –20 °C

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.[2,3] ELISA applications that use pNPP may be read in timed assays or stopped with alkaline solutions for delayed readings.[4] 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.

Several publications[5-8] and theses[9,10] have cited use of Product Number N3129 in their research protocols.

Precautions and Disclaimer

This product is for R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Dissolve pNPP in either:

- 0.1 M glycine buffer that contains 1 mM $MgCl_2$, 1 mM $ZnCl_2$, pH 10.4, or
- 1 M 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.

Procedure

General ELISA procedure with alkaline phosphatase conjugates

- Add 200 µL of substrate solution (typically 1 mg/mL) per well.
- 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 adding 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:
   - 0.05% TWEEN® 20 in 50 mM TBS, pH 8.0.
   - 1% BSA containing 0.05% TWEEN® 20 in 50 mM TBS, pH 8.0.
   - 3% nonfat-dried milk in 0.01 M TBS (Cat. 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

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