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Product Information

p-Nitrophenyl Phosphate Liquid Substrate System

Catalog Number **N7653**
Storage Temperature –20 °C

CAS RN 4264-83-9
Synonyms: 4-Nitrophenyl phosphate disodium salt solution, pNPP

Product Description

In ELISA experiments that use alkaline phosphatase, *p*-nitrophenylphosphate (pNPP) is a widely used substrate.[1-2] The *p*-Nitrophenyl Phosphate Liquid Substrate System combines pNPP, buffer, and the required magnesium cations in a convenient, ready-to-use, single solution reagent. It is recommended for ELISA procedures.

This product demonstrates a high sensitivity for the detection of alkaline phosphatase activity. This liquid is ready-to-use and does not require the addition of any further ingredients or preparatory steps. The soluble yellow end product absorbs at 405 nm. The reaction may be stopped with the addition of 3 M NaOH solution. The absorbance of the stopped reaction is read at 405 nm. For ELISA applications, typically 200 µL of substrate is added per well of the ELISA plate, and the reaction is stopped with 50 µL of 3 M NaOH solution.

Several publications cite use of this product.[5-10]

Precautions and Disclaimer

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.

Procedure

This product is supplied as a ready-to-use solution. No additional ingredients need to be added before use.

1. After incubating the plate with an alkaline phosphatase conjugate (generally 1–2 hours), wash thoroughly to remove unbound conjugate.
2. Add 200 µL of pNPP solution to each well. Incubate the plate in the dark for ~30 minutes at room temperature.
3. After the incubation period, read the plate at 405 nm on a multiwell plate reader.
4. If the plate cannot be read immediately, add 50 µL of 3 M NaOH solution per 200 µL of reaction mixture. Read the absorbance for the stopped reactions at 405 nm.
5. Dispose of any remaining substrate solution.

<u>Troubleshooting</u>

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 (e.g., Catalog Number 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 the 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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