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Product Information

SIGMA*FAST*™ *p*-Nitrophenyl phosphate Tablets
tablet, to prepare 5 mL

Catalog Number **N1891**
Storage Temperature –20 °C

Synonym: pNPP

Product Description

SIGMA*FAST*™ *p*-Nitrophenyl phosphate (pNPP) tablets have been developed for use as a soluble substrate for the detection of alkaline phosphatase activity in Enzyme Immunoassays (EIA and ELISA assays).[1,2] pNPP is the EIA/ELISA substrate of choice in alkaline phosphatase systems, as it exhibits high sensitivity. EIA/ELISA applications utilizing pNPP may be read in timed assays, or stopped with alkaline solutions for delayed readings. SIGMA*FAST* pNPP tablets require no additional buffers to prepare an active substrate solution.

One pNPP tablet and one Trizma® Buffer tablet, dissolved in 5 mL of water, provides 5 mL of ready-to-use substrate. Each SIGMA*FAST* pNPP tablet set yields 5 mL of a solution containing:

1.0 mg/mL pNPP
0.2 M Trizma buffer
5 mM magnesium chloride

Various publications have cited use of this product to report alkaline phosphate activity in ELISA,[3-7] as well as in binding assays,[8] and studies of hydrogels[9] and of receptors.[10]

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

1. Remove the required number of pNPP and Trizma Buffer tablets for assay. Return the box to the freezer.
2. Allow the tablets to warm to room temperature.
3. Open an equal number of pNPP tablet packages (silver foil) and Trizma Buffer tablet packages (gold foil).
4. Drop the tablets into an appropriate container containing 5 mL of water for each tablet set. **Do not touch the tablets with your fingers.**
5. Vortex the solution until the tablets completely dissolve.

The SIGMA*FAST* pNPP substrate solution is now ready for use. For best results, the solution should be used within one hour.

Procedure

1. After the plate has been incubated with an alkaline phosphatase conjugate (generally 1–2 hours), wash thoroughly to remove unbound conjugate.
2. Add 200 µL of pNPP substrate 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 N NaOH solution per 200 µL of reaction mixture.
5. Read the absorbance for the stopped reactions at 405 nm.

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 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 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 amplification system such as avidin-biotin.

References

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