

# pNPP Tablets

## p-Nitrophenyl phosphate Tablets

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Product	Cat#	Package size
pNPP Tablets (5mg)	D2010.0024	24 tablets
pNPP Tablets (5mg)	D2010.0100	100 tablets
pNPP Tablets (20mg)	D2011.0024	24 tablets
pNPP Tablets (20mg)	D2011.0100	100 tablets

### Product description

p-Nitrophenyl phosphate (pNPP) is a soluble substrate for the detection of alkaline phosphatase activity in Enzyme Immuno assays (EIA and ELISA). For easier handling pNPP tablets have been developed to be used as ready-to-use in preparing EIA or ELISA tests. pNPP is the substrate of choice in alkaline phosphatase systems as used in many EIA/ELISA applications with high sensitivity. EIA/ELISA applications utilizing pNPP may be read in timed assays or stopped with alkaline solutions for delayed readings. One pNPP tablet dissolved in 5mL (D2010) or 20mL (D2011) buffer yields a solution of 1mg/mL pNPP.

### Procedure

- Remove the required number of pNPP tablets for assay and return the box to the freezer. Allow tablets to warm to room temperature. Dissolve tablets in 5mL (D2010) or 20mL (D2011) of one of the following buffers: a. 0.1M glycine, pH10.4, 1mM MgCl<sub>2</sub>, 1mM ZnCl<sub>2</sub>. b. 1M diethanolamine, pH9.8, 0.5mM MgCl<sub>2</sub>. c. 0.2M Tris, pH8.8, 5mM MgCl<sub>2</sub>. **NOTE:** Do not touch the tablets with your fingers. Vortex the solution until the tablets completely dissolve. **NOTE:** for best results, the solution should be used within one hour.
- Preparation of buffer:
  - Add 7.51g glycine, 203mg MgCl<sub>2</sub> and 136mg ZnCl<sub>2</sub> to 900mL water and mix. Adjust pH to 10.4 with conc. NaOH solution and adjust volume to 1L with water.
  - Add 97mL diethanolamine and 100mg MgCl<sub>2</sub> to 800mL water. Adjust pH to 9.8 with 10M HCl solution and adjust volume to 1L with water.
  - Add 24.23g Tris (free base) and 1.02g MgCl<sub>2</sub> to 900mL water and mix. Adjust pH to 8.8 with 10M HCl solution and adjust volume to 1L with water.
- After the plate has been incubated with an alkaline phosphatase conjugate (generally 1-2 hours), wash thoroughly to remove unbound conjugate.
- Add 200µL of pNPP substrate solution to each well. Incubate the plate in the dark for approximately 30 minutes at room temperature.
- After the incubation read the plate at 405nm on a multiwell plate reader.
- If the plate cannot be read immediately, add 50µL of 3M NaOH solution per 200µL of reaction. Read the absorbance for the stopped reactions at 405nm at any time.
- Dispose of any remaining substrate solution.

### Trouble shooting

If the background is too high

- 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.
- Additionally blocking agents for ELISA are:
  - 0.05% Tween®20 in 50mM TBS, pH8.0.
  - 1% BSA containing 0.05% Tween®20 in 50mM TBS, pH8.0.
  - 3% nonfat-dried milk in 0.01M TBS. Do not use milk as a blocking agent when using avidin-biotin systems.
- Use 0.05% Tween®20 in all washing and antibody diluents buffers.
- Run control wells without the primary antibody to check for non-specific reactivity of the secondary antibody/alkaline phosphatase conjugate.
- Adjust the titer of the primary antibody and/or the alkaline phosphatase conjugate to determine the optimal working dilutions.

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### Trouble shooting (continued)

If no colour develops or colour is too faint

1. Adjust concentration of the primary antibody.
2. Adjust 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 concentration of the coating antigen.
6. Consider using an amplification system such as avidin-biotin.