

pNPP Substrate

A one-component formulation suitable for all ELISAs using AP.

pNPP Substrate is suitable for use in all ELISAs where alkaline phosphatase (AP) is the conjugated detection enzyme and the target detection level is in the ng-pg/mL range. pNPP Substrate should not be used for membrane or immunohistochemical applications.

pNPP Substrate is a one-component, ready-to-use substrate formula-tion containing p -nitrophenylphosphate and stabilizing pellets. pNPP Substrate does not contain any aprotic solvents. The pNPP substrate is oxidized by the alkaline phosphatase enzyme to yield a soluble, colorless p -nitrophenol intermediate product. In alkaline pH environments (pH > 8), the colorless intermediate converts to the bright yellow p -nitrophenolate reaction product, which can be read between 405-420 nm. The reaction can be stopped by adding equal volumes of Stop Solution (AP) (catalog# LS-M44). Addition of Stop Solution (AP) does not change the color of the chromagen and stabilizes the reaction for one hour.

For best results, the absorbance should be monitored and read before values exceed 2.5 OD units. The substrate should not be diluted. If the reaction yields OD values above 2.5 units, it is recommended to dilute the conjugate or shorten the conjugate incubation period.

pNPP Substrate is ready to use at 1X; add 100 µL to each well. Best results are obtained by equilibrating the pNPP substrate to room temperature (25°C) prior to use.

pNPP 1-SUBSTRATE

Size	Catalog #
100 mL	LS-M39-100

INSTRUCTIONS:

1. Run ELISA according to the specific protocol through the conjugate incubation step.
2. Wash the wells three or four times with 1X ELISA Wash Buffer (catalog# LS-M27) to remove any residual AP-conjugate.
3. Bring pNPP Substrate to room temperature; protect from light.
4. Pipette 100 µL pNPP Substrate into each well of the plate.
5. Incubate pNPP Substrate 10-60 minutes. Monitor the color intensity.
6. Read the plate between 405-420 nm and analyze. Alternatively, stop the reaction by adding 100 µL/well Stop Solution (AP) and read the samples between 405-420 nm within 1 hour.

For more ELISA protocols and information, please visit www.LSBio.com.

SPECIFICATIONS:

- Colorless to light yellow liquid with stabilizing pellets
- 1X ready to use
- Read absorbance between 405-420 nm.
- Use Stop Solution (catalog# LS-M44) to stabilize the reaction and read between 405-420 nm.

STORAGE:

- 2-8°C
- Protect from light

SAFETY & USAGE:

- Contains Diethanolamine and Hydrogen chloride.
- SDS available upon request
- Not for human or drug use
- For research use only



2401 4th Avenue, Suite 900, Seattle, WA 98121

USA TOLL-FREE 1-866-819-4732 LOCAL 206-374-1102

www.LSBio.com