F17-3328-3-D; Effective 06/08/2017 ; Supersedes F17-3328-3-C

pNPP 1-Component AP Microwell Substrate with Stabilizing Pellets (SUBP)

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

pNPP 1-Component AP Microwell Substrate with Stabilizing Pellets (SUBP) 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. SUBP should not be used for membrane or immunohistochemical applications.

SUBP is a one-component, ready-to-use substrate formulation containing ρ -nitrophenylphosphate and stabilizing pellets. SUBP does not contain any aprotic solvents. The pNPP substrate is oxidized by the alkaline phosphatase enzyme to yield a soluble, colorless ρ -nitrophenol intermediate product. In alkaline pH environments (pH > 8), the colorless intermediate converts to the bright yellow ρ -nitrophenolate reaction product, which can be read between 405-420 nm. The reaction can be stopped by adding equal volumes of Stop Solution for pNPP Microwell Substrates (STOPP, catalog #6284). Addition of STOPP 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 1-Component AP Microwell Substrate with Stabilizing Pellets 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-COMPONENT AP MICROWELL SUBSTRATE with STABILIZING PELLETS (SUBP)

Size	Catalog #
100 mL	#6279

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 #652) to remove any residual AP-conjugate.
3. Bring SUBP to room temperature; protect from light.
4. Pipette 100 µL SUBP into each well of the plate.
5. Incubate SUBP 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 STOPP (catalog #6284) and read the samples between 405-420 nm within 1 hour.

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

SPECIFICATIONS:

- Colorless to light yellow liquid with stabilizing pellets
- 1X ready to use
- Read absorbance between 405-420 nm.
- Use STOPP 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 at immunochemistry.com
- Not for human or drug use
- For research use only

Build a better assay with ELISA Solutions from ImmunoChemistry Technologies.

ImmunoChemistry TECHNOLOGIES LLC

BRIGHT MINDS, BRIGHT SOLUTIONS.

ImmunoChemistry Technologies, LLC gratefully acknowledges the significant contributions made by one of its founders, Brian W. Lee, Ph.D in the development of this product, including the creation and illustration of its strategy and protocol.

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