

Product Datasheet

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pNPP Alkaline Phosphatase Microwell Substrate One Component “Ready Use”

Prod. No.: P162

Pkg. Size: 100 ml, 500 ml, 1 L

Storage: 2 – 8°C *Detailed storage instructions below.*

MSDS

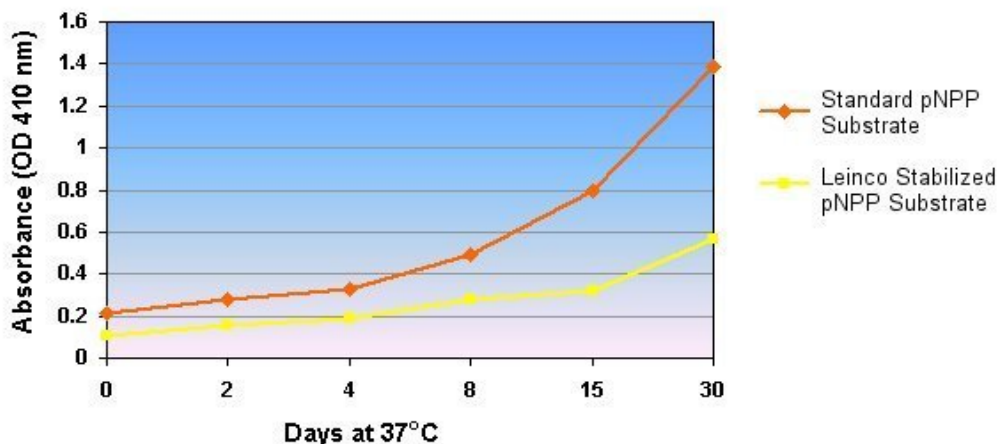
Description

pNPP Alkaline Phosphatase Microwell Substrate (*p*-nitrophenylphosphate) is a soluble substrate used with the enzyme alkaline phosphatase designed for various qualitative or quantitative immunoassays but not recommended for membrane or immunohistochemical applications where a precipitating reaction product is required. The substrate is supplied as a one component ready to use solution. Initially the substrate should be colorless to pale yellow in appearance. When this substrate system is reacted with alkaline phosphatase, a soluble, yellow product is obtained.

Storage and Stability

The high quality of the substrate can be preserved by storing at temperatures between 2 – 8°C. When properly stored, pNPP Alkaline Phosphatase Substrate is stable for a minimum of 48 months from the manufactured date. The substrate performance has been shown to be within specifications following 5 freeze-thaw cycles, however freezing is not recommended. The substrate should be protected from direct light by storing in amber bottles. Only high quality glass and plastic products should be used for storing aliquots.

Leinco Technologies' pNPP Background Stability Study



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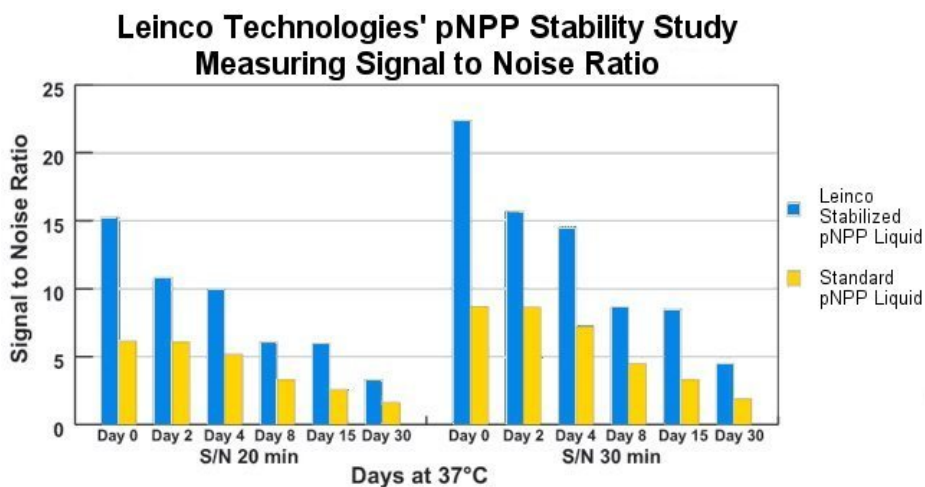
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Directions for Product Use

pNPP Alkaline Phosphatase Microwell Substrate is a ready to use solution that needs no preparation or dilution. Pour estimated amount of substrate into a suitable high quality plastic reservoir to avoid contamination of the bulk solution. It is recommended that you allow the substrate solution to equilibrate to room temperature before use. While the pNPP solution is equilibrating, wash the microplates thoroughly to remove excess alkaline phosphatase labeled conjugates. Washing the plates at least four times is recommended to minimize background noise.

For immunoassay microwell applications 100 – 200 μ l of substrate solution is added to each well producing a soluble, yellow product. For best results, sample OD values should be monitored and read before absorbance values exceed 2.0 OD units. The substrate reaction can be stopped using 50 μ l of pNPP Stop Solution ([Leinco Prod. No.: P227](#)) or 50 μ l of 3N NaOH per 200 μ L of substrate. Sample OD values are read, with or without the addition of stop solution, in the 405-420nm range. Dilution of the substrate is not recommended. To reduce the intensity of a reaction, it is recommended that the antibodies or conjugates be diluted. This substrate is light sensitive and should be protected from direct sunlight or UV sources.



Reported Applications

pNPP Alkaline Phosphatase Microwell Substrate is suitable for use in ELISA based assays

Country of Origin

USA

Related Products

pNPP Stop Solution ([Leinco Prod. No.: P227](#))

UltraAvidin™-Alk. Phos. ([Leinco Prod. No.: A108](#))

Streptavidin- Alk. Phos. ([Leinco Prod. No.: S208](#))

Goat Anti-Mouse IgG (H&L)- Alk. Phos. ([Leinco Prod. No.: M290](#))

Goat Anti-Human IgG (H&L)-Alk. Phos. ([Leinco Prod. No.: H627](#))

Goat Anti-Rat IgG (H&L)-Alk. Phos. ([Leinco Prod. No.: R1216](#))

Goat Anti-Rabbit IgG (H&L)-Alk. Phos. ([Leinco Prod. No.: R1191](#))

Goat Anti-Armenian Hamster IgG (H&L)-Alk. Phos. ([Leinco Prod. No.: A130](#))

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References

1. Robey, F.A. *et al.* (2001). *AIDS Res. Hum. Retroviruses*. **17**:533.

Troubleshooting Guide

Problem	Possible Causes	Possible Solutions
High Background Noise	Insufficient plate blocking Step	<ul style="list-style-type: none">• 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.• Additional blocking agents for ELISA are:<ul style="list-style-type: none">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 (Do not use milk as a blocking agent when using avidin-biotin systems)
	Insufficient plate washing	<ul style="list-style-type: none">• Add 0.05% TWEEN 20 in all washing and antibody diluent buffers• Increase number of washes
	Concentration of primary antibody and/or alkaline phosphatase conjugate is too high	<ul style="list-style-type: none">• Adjust the titer of the primary antibody and/or the alkaline phosphatase conjugate to determine the optimal working dilutions.
	Non-specific secondary antibody binding	Run control wells without the primary antibody to check for non-specific reactivity of the secondary antibody/alkaline phosphatase conjugate.
No/Low Signal	Capture antibody did not bind to plate	<ul style="list-style-type: none">• Evaluate coating conditions and standardize• Increase coating time• Increase coating concentration• Change plate type to high binding
	Contaminated buffers or incorrect solutions	Repeat assay with fresh buffers and solutions
	Not enough reporter antibody used	Increase concentration of alkaline phosphatase labeled secondary antibody
	Enzyme conjugate has expired	Determine if the enzyme conjugate is active by mixing a small sample of substrate and conjugate together in a test tube.
	Insufficient substrate incubation and/or temperature	Increase the substrate incubation time or temperature.
	Insufficient amplification	Consider using an amplifying system such as avidin-biotin.