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# Product Information

**Citrate Buffer Solution**
**0.09 M, pH 4.8 (25 °C), and contains 1% chloroform**

Catalog Number **C2488**
Storage Temperature 2–8 °C

## Product Description

Citrate buffer solution, 0.09 M, pH 4.8 at 25 °C is for use in acid phosphatase reactions. It is used in conjunction with *p*-nitrophenyl phosphate enzyme substrate.

*p*-Nitrophenyl phosphate (pNPP) is a soluble substrate for use with alkaline phosphatase conjugates in ELISA procedures. It may also be used for the determination of alkaline and acid phosphatase activity in physiological fluids and other aqueous solutions. This substrate produces a soluble end product that is yellow in color and can be read spectrophotometrically at 410 nm. The pNPP reaction may be stopped with 0.1 M NaOH solution and read at 410 nm.

Unit definition: One unit of acid phosphatase will hydrolyze 1.0 μmole of *p*-nitrophenyl phosphate per minute at pH 4.8 at 37 °C.

## Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

## Preparation Instructions

Prepare a stock solution of pNPP substrate at a concentration of 4 mg/ml in water. Use immediately or dispense in 0.5 ml aliquots and store at –20 °C. Stock solution is stable for 6 weeks when stored at –20 °C.

## Storage/Stability

Store the product at 2–8 °C.

## Procedure

1. Add 0.5 ml of pNPP substrate solution (4 mg/ml) to each of two tubes. If using frozen aliquots, equilibrate tubes in 37 °C water bath.
2. Pipette 0.5 ml of Citrate Buffer Solution, 0.09 M, pH 4.8 (25 °C), (Catalog Number C2488) into each tube. Mix and equilibrate in 37 °C water bath.
3. Pipette 0.2 ml of water into one tube. Label as Reagent Blank.
4. Pipette 0.2 ml of serum or sample containing acid phosphatase into second tube. Label tube as Test. Record exact time sample was added to the second tube. Mix gently and return to water bath for 30 minutes.
5. Prepare Sample Blank by adding 0.2 ml of serum or sample containing acid phosphatase into a third tube. Add 6.0 ml of 0.1 N NaOH to the Sample Blank. Stopper and mix by inversion.
6. Exactly 30 minutes after adding serum or sample containing acid phosphatase to the pNPP substrate in step 4, stop the reaction by pipetting 5.0 ml of 0.1 N NaOH to the Test and Reagent Blank. Stopper and mix by inversion. The sodium hydroxide stops the enzymatic reaction and develops the color of the *p*-nitrophenol which is stable several hours.
7. Read the absorbance of the Sample Blank solution versus water at 410 nm ($\Delta A_{410}$ Blank).
8. Read the absorbance of the Test solution versus the Reagent Blank solution at 410 nm ($\Delta A_{410}$ Test).

**Results**

Calculations

Acid phosphatase activity is calculated using the formula:

$$\text{Units/ml serum or sample} = \frac{(\Delta A_{410}\ \text{Test} - \Delta A_{410}\ \text{Blank})\ (6.2)}{(30)\ (18.3)\ (0.2)}$$

Where:
6.2 = Total volume of reaction in ml
30 = Time of reaction in minutes
18.3 = Millimolar absorptivity (extinction coefficient) of *p*-nitrophenol at 410 nm at alkaline pH
0.2 = Initial volume of serum or acid phosphatase sample used in ml

Alternatively, a standard curve of *p*-nitrophenol may be prepared using multiple dilutions. Dilute with 0.1 N NaOH to develop color and read at 400–420 nm. Read all Test and Blank solutions at same wavelength as the standard curve solutions.

**Related Products**

Sigma-Aldrich offers a selection of powdered and tableted forms of pNPP phosphatase substrate. Visit the Enzyme Explorer on line for more details:

sigma-aldrich.com/enzymeexplorer

4-Nitrophenol (*p*-nitrophenol) is the hydrolysis product of *p*-nitrophenyl phosphate (pNPP) and may be used to prepare a standard curve to determine enzyme activity instead of using the millimolar absorptivity value. It has a formula ($C_6H_5NO_3$) weight of 139.11.

10 mM 4-Nitrophenol solution (Catalog No. N7660) is available for use in preparing a standard curve of *p*-nitrophenol.

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