

Product Information

Alkaline Buffer Solution

1.5 M, pH 10.3 (25 °C)

Catalog Number **A9226**

Storage Temperature 2–8 °C

Product Description

Alkaline buffer solution, 1.5 M, pH 10.3 at 25 °C is for use in alkaline phosphatase reactions. It contains 2-amino-2-methylpropanol (AMP) and is used in conjunction with *p*-nitrophenyl phosphate (pNPP) enzyme substrate.

p-Nitrophenyl phosphate 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 0.05 M NaOH solution and read at 410 nm.

Unit definition: One unit of alkaline phosphatase will hydrolyze 1.0 μ mole of *p*-nitrophenyl phosphate per minute at pH 10.3 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 Alkaline Buffer Solution, 1.5 M, pH 10.3 (25 °C), (Catalog Number A9226) into each tube. Mix and equilibrate in 37 °C water bath.
3. Pipette 0.1 ml of water into one tube. Label tube as Blank.
4. Pipette 0.1 ml of serum or sample containing alkaline phosphatase into second tube. Label tube as Test. Record exact time sample was added to the second tube. Mix gently and return to 37 °C water bath for 15 minutes.
5. Exactly 15 minutes after adding serum or sample containing alkaline phosphatase to the pNPP substrate in step 4, stop the reaction by pipetting 10.0 ml of 0.05 N NaOH to the Test and Blank tubes. Stopper and mix by inversion. The sodium hydroxide solution stops the enzymatic reaction and develops the color of the *p*-nitrophenol, which is stable several hours.
6. Read the absorbance of the Test solution versus the Blank solution at 410 nm (ΔA_{410} Total).

Note: If using serum or other sample, which has an intrinsic absorbance at 410 nm, include steps 7 and 8.

7. Add 0.2 ml of concentrated HCl to each tube and mix. The acid removes the color due to *p*-nitrophenol, leaving the intrinsic absorbance due to the sample added to the reaction.
8. Read the absorbance of the Test solution versus the Blank solution at 410 nm (ΔA_{410} Background).

Results

Calculations

Alkaline phosphatase activity is calculated using the formula:

$$\text{Units/ml serum or sample} = \frac{(\Delta A_{410} \text{ Total}) (11.1)}{(15) (18.3) (0.1)}$$

If using the procedure which includes treatment with hydrochloric acid (steps 7 and 8):

$$\text{Units/ml serum or sample} = \frac{(\Delta A_{410} \text{ Total} - \Delta A_{410} \text{ Background}) (11.1)}{(15) (18.3) (0.1)}$$

Where:

11.1 = Total volume of reaction in ml

15 = Time of reaction in minutes

18.3 = Millimolar absorptivity (extinction coefficient) of *p*-nitrophenol at 410 nm at alkaline pH

0.1 = Initial volume of serum or alkaline phosphatase sample used in ml

Alternatively, a standard curve of *p*-nitrophenol may be prepared using multiple dilutions. Dilute with 0.05 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 (C₆H₅NO₃) 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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