Technical Bulletin

Acid Phosphatase Assay Kit

Catalog Number CS0740

Product Description

Acid Phosphatase is one of the acid hydrolases that normally reside in lysosomes. It is a classical marker for the identification of lysosomes in subcellular fractionations.

The Acid Phosphatase Assay Kit is designed for the detection of acid phosphatase activity in whole cell and tissue extracts, column fractions, and purified enzyme preparations. The kit contains all the reagents required for fast and simple acid phosphatase detection. The kit contains a standard solution and a control enzyme.

Components

The kit is sufficient for 100 assays in tubes or 1,000 assays in 96-well plates.

- 4-Nitrophenyl Phosphate Tablets 20 tablets
  Catalog Number N9389
- Citrate Buffer Solution, 0.09 M, pH 4.8 100 mL
  Catalog Number A4855
- p-Nitrophenol Standard Solution, 10 mM 1 mL
  Catalog Number N7660
- Acid Phosphatase Control Enzyme 0.2 mL
  Catalog Number P3248

Reagents and Equipment Required but Not Provided

- Sodium hydroxide (NaOH; Catalog Number S5881 or equivalent)
- Spectrophotometer or ELISA reader
- Cuvettes (3 ml, Catalog Number C5291), or 96 well plates (flat bottom, Catalog Number P7366)
- 37 °C water bath

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Use ultrapure water (17 MΩ-cm or equivalent) for preparation of reagents.

Substrate Solution – For 5 assays in tubes, completely dissolve one 4-Nitrophenyl Phosphate Tablet (Catalog Number N9389) in 5 ml of the Citrate Buffer Solution (Catalog Number A4855). For 50 assays in a 96 well plate, dissolve one 4-Nitrophenyl Phosphate Tablet in 2.5 ml of the Citrate Buffer Solution. The Substrate Solution should be freshly prepared.

Stop Solution (**not supplied**) – Prepare a 0.5 N NaOH solution by dissolving 2 g of NaOH (Catalog Number S5881 or equivalent) in 100 ml of ultrapure water.

Standard Solution – For the preparation of 1 ml of standard solution, dilute 5 µl of the 10 mM *p*-Nitrophenol Standard Solution (Catalog Number N7660) in 995 µl of the Stop Solution (0.5 N NaOH). The diluted Standard Solution allows quantitative results to be obtained when performing the assay in 96 well plates. (1 ml of the diluted Standard Solution is sufficient for 3 standard measurements in a 96 well plate)

Storage/Stability

The kit is shipped on dry ice and storage at -20 °C is recommended.

Citrate Buffer Solution - Cloudiness may develop due to changes in temperature and chloroform globules often settle out. This does not affect the quality of the reagent but avoid pipetting chloroform globules.

Procedure

I. Assay in Tubes (3 ml final volume)

1. Equilibrate the Substrate Solution to 37 °C by incubating for several minutes.
2. Set the spectrophotometer at 405 nm.
3. Add the reaction components to the tubes according to Table 1 and vortex the tubes briefly.

Table 1.
Reaction Scheme for Tube Assays

	Substrate Solution	**Sample**
Test Sample	900–990 µl	10-100 µl of test sample
Reagent Blank*	1 ml	–
Positive Control	990 µl	10 µl of Acid Phosphatase Control Enzyme

* A Reagent Blank reaction (Substrate Solution without enzyme) should be run in parallel to account for the 4-nitrophenyl phosphate that will hydrolyze spontaneously during the incubation time.

4. Incubate the tubes for 5 minutes at 37 °C. For the Positive Control, a 10 minute incubation should be performed.

   If you suspect that the acid phosphatase activity of the Test Sample is low, the incubation time can be extended up to 30 minutes.

5. Stop the reactions by adding 2 ml of Stop Solution to each tube. The colored solution formed after the addition of the 0.5 N NaOH is stable for several hours.
6. Transfer the reaction mixture to a cuvette and measure the absorption (A) at 405 nm.

II. 96-Well Plate Assay

Note: It is recommended to perform the assays in triplicate.

1. Equilibrate the Substrate Solution to 37 °C by incubating for several minutes.
2. Set the plate reader at 405 nm.
3. Add the reaction components to the 96-well plates according to Table 2 and mix using a horizontal shaker or by pipetting.

Table 2.
Reaction Scheme for 96-Well Plate Assays

	Substrate Solution	**Sample**	**Citrate Buffer**	**Standard Solution**
Test Sample	50 µl	50 µl of test sample	-	-
Reagent Blank*	50 µl	-	50 µl	
Standard	-	-	-	300 µl
Positive Control	50 µl	2 µl of Control Enzyme	48 µl	-

* A Reagent Blank reaction (Substrate Solution without enzyme) should be run in parallel to account for the 4-nitrophenyl phosphate that will hydrolyze spontaneously during the incubation time.

4. Incubate the plate for 5-10 minutes at 37 °C.

   If you suspect that the acid phosphatase activity of the Test Sample is low, the incubation time can be extended up to 30 minutes.

5. Stop the reactions by adding 200 µl of Stop Solution to each well, **except** to wells containing the Standard Solution. The colored solution formed after the addition of the 0.5 N NaOH is stable for several hours.
6. Measure the absorption (A) at 405 nm.

Results

Calculate the acid phosphatase activity in the sample according to the following equations:

3 ml Cuvette Assay

Units/ml =

$$\frac{(A_S - A_B) \times 3 \times DF}{18.3 \times T \times V}$$

96-Well Plate Assay

Units/ml =

$$\frac{(A_S - A_B) \times 0.05 \times 0.3 \times DF}{A_{STD} \times T \times V}$$

where:

A_S = Absorbance of the Test Sample at 405 nm

A_B = Absorbance of the Blank at 405 nm

A_{STD} = Absorbance of the Standard Solution at 405 nm

3 = Final volume in milliliters in the reaction cuvette after addition of Stop Solution

DF = Enzyme dilution factor (DF = 1 for undiluted samples)

18.3 = Millimolar extinction coefficient for *p*-nitrophenolate ion

T = Reaction time in minutes

V = Volume of the Test Sample in milliliters

0.05 = Concentration (µmole/mL) of *p*-nitrophenol in the Standard Solution (96-well plate assay)

0.3 = Final volume in milliliters of the 96-well plate reaction after addition of the Stop Solution

Unit definition: One unit of acid phosphatase will hydrolyze 1 µmole of 4 Nitrophenyl phosphate per minute at pH 4.8 at 37 °C.

Reference

Bergmeyer, H.U., et al., in Methods of Enzymatic Analysis, Volume I, 2nd ed., Academic Press, Inc., (New York, NY: 1974) pp.495-496.

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