



Product Information

Acid Phosphatase Assay Kit

Catalog Number **CS0740**
Storage Temperature $-20\text{ }^{\circ}\text{C}$

TECHNICAL BULLETIN

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 C2488

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

- NaOH (Catalog Number S5881)
- 0.5 N NaOH solution
- Spectrophotometer or ELISA reader
- Cuvettes (3 ml, Catalog Number C5291), or 96 well plates (flat bottom, Catalog Number P7366)

Precautions and Disclaimer

The kit is for R&D use only, not for drug, household or other uses. Please refer to the Material Safety Data Sheet (MSDS) for information regarding hazards and safe handling practices.

Preparation Instructions

Use ultrapure water (17 M Ω -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 C2488). 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) in 100 ml of ultrapure water.

Standard Solution – For the preparation of 1 ml of standard solution, dilute 5 μ l of the 10 mM *p*-Nitrophenol Standard Solution (Catalog Number N7660) in 995 μ l 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 $-20\text{ }^{\circ}\text{C}$ is recommended.

Procedures

Assay in Tubes (3 ml final volume)

1. Equilibrate the Substrate Solution to 37 °C.
2. Set the spectrophotometer at 405 nm.
3. Add the reaction components to the tubes according to the scheme in Table 1.

Table 1.

Reaction Scheme for Tube Assays

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

4. Vortex the tubes briefly. 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.

A 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.

5. Stop the reactions with 2 ml of Stop Solution. 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 at 405 nm.

96 Well Plate Assay

It is recommended to perform the assays in triplicates.

1. Equilibrate the Substrate Solution to 37 °C.
2. Set the plate reader at 405 nm.
3. Add the reaction components to the 96 well plate according to the scheme in Table 2.

Table 2.

Reaction Scheme for 96 Well Plate Assay

	Substrate Solution	Sample	Citrate Buffer	Standard Solution
Test	50 µl	50 µl of test sample	-	-
Blank	50 µl	-	50 µl	-
Standard	-	-	-	300 µl
Positive Control	50 µl	2 µl of control enzyme	48 µl	-

4. Mix using a horizontal shaker (or by pipetting) and 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.

A 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.

5. Stop the reactions by adding 0.2 ml of Stop Solution to the wells, except for the 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 at 405 nm.

Results

Calculations

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

3 ml Cuvette Assay

$$\text{Units/ml} = \frac{(A_{405} [\text{sample}] - A_{405} [\text{blank}]) \times 3 \times \text{DF}}{18.3 \times \text{Time} \times \text{Venz}}$$

96 Well Plate Assay

$$\text{Units/ml} = \frac{(A_{405}[\text{sample}] - A_{405}[\text{blank}]) \times 0.05 \times 0.3 \times \text{DF}}{A_{405}[\text{standard}] \times \text{Time} \times \text{Venz}}$$

Where:

A₄₀₅ [sample] = the absorbance of the sample

A₄₀₅ [blank] = the absorbance of the blank

A₄₀₅ [standard] = the absorbance of the standard

DF = dilution factor of the original sample

Time = time of incubation at 37 °C in minutes

Venz = volume of enzyme sample added to the assay in ml.

18.3 = millimolar extinction coefficient (ϵ^{mM}) of 4-nitrophenol at 405 nm

0.05 = concentration ($\mu\text{mole/ml}$) of 4-nitrophenol in the standard solution (for the assay in 96 well plate)

0.3 = 0.3 ml, the total assay volume in 96 well plate, including the Stop Solution

3 = 3 ml, the total assay volume in the cuvette, including 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.

References

1. 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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