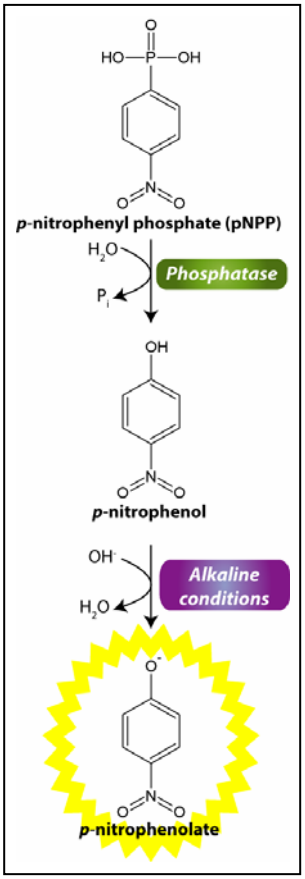
Phosphatase Assay Kit



INTRODUCTION

The Phosphatase Assay kit is designed to measure the activity of phosphatases in biological samples and to screen for agonists and inhibitors of phosphatases. The Phosphatase Assay kit uses *para*-nitrophenyl phosphate (*p*NPP), a chromogenic substrate for most phosphatases, including alkaline phosphatases, acid phosphatases, protein tyrosine phosphatases and serine/threonine phosphatases (Figure 1). The phosphatases remove the phosphate group to generate p-nitrophenol, which is deprotonated under alkaline conditions to produce p-nitrophenolate that has strong absorption at 405nm. The kits components are sufficient for performing up to 1000 assays in 96-well plate format and easily adaptable to cuvettes or 384-well plates.

ITEM SUPPLIED **Cat # 786-453**

PA Substrate	50ml
PA Assay Buffer	50ml

STORAGE

Shipped at ambient temperature. Upon arrival, store at 4°C. The substrate is light sensitive and should be protected from direct sunlight or UV sources.

ITEM NEEDED BUT NOT SUPPLIED

Stop Solution: 3N Sodium hydroxide (optional).

PROTOCOL

NOTE: The pH of the supplied assay buffer is 7.5, which is compatible with a large percentage of phosphatases, however for improved assay results use the following recommended assay buffers for acid and alkaline phosphatases.

***Acid Phosphatases**: 0.1M sodium acetate (pH5.5) and 10mM $MgCl_2$*

***Alkaline Phosphatase**: 0.1M Tris.HCl (pH 8.6) and 10mM $MgCl_2$*

Equilibrate the substrate and assay buffer to room temperature before use.

1. Make appropriate serial dilutions of your test samples with PA Assay Buffer. Prepare enough solution to perform assays in triplicate.

2. Transfer 50µl diluted test samples to wells in a 96-well plate. In addition, use 50µl PA Assay Buffer without enzyme for blank wells.

3. Start the reaction with the addition of 50µl PA Substrate to each well.

4. Incubate for 10-30 minutes at room temperature.
   NOTE: For dynamic assay, measure the absorbance every minute at 405nm.

5. At the end of the incubation, add 50µl Stop Solution to each well to stop the reaction.

6. Measure the absorbance at 405nm.

Enzyme Activity Calculation:

Calculate the average of the triplicate assays and subtract the average of the blank wells.

Enzyme activity (nmol/min/μg) = (OD_{405nm} x V) / (ε x T x L x E)

OD_{405nm} = mean absorbance of sample minus mean absorbance of blank
V = reaction volume (μl)
ε = extinction coefficient of p-nitrophenol (17.8 $nmol^{-1}cm^{-1}$)
T = Incubation time (min)
L = pathlength of light (cm)
E = enzyme (μg)

RELATED PRODUCTS

1. ***AP-Labeled Secondary Antibodies***

Cat. #	*Description*	*Size*
786-R43	*Goat ant-Mouse IgG H&L-AP*	*1ml*
786-R44	*Goat anti-Rabbit IgG H&L-AP*	*1ml*
786-R45	*Goat anti-Rat IgG H&L-AP*	*1ml*
786-R46	*Goat anti-Human IgG H&L-AP*	*1ml*
786-R47	*Rabbit anti-Goat IgG H&L-AP*	*1ml*
786-R49	*Rabbit anti-Human IgG H&L-AP*	*1ml*

2. ***NAP-Blocker (2X) Cat # 786-190***
A Non-Animal Protein, suitable for use as blocking agent in ELISA plates, PVDF and Nitrocellulose membranes.

3. ***femtoTBST, 10X (Cat # 786-161) and femtoPBST, 10X (Cat # 786-162):*** *These buffers are supplied as 10X concentrate, to use as wash buffers in ELISA and Western protocols.*

4. ***Non-Interfering (NI) Protein Assay™ Cat. # 786-005***
Interfering agents commonly present in the protein solutions do not affect NI-Protein Assay™. The assay shows no protein-to-protein variation also. The NI-Protein Assay™ is based on removal of interfering agents, prior to assay by a single step protocol. Sensitivity is as low as 0.5ug/assay and assay time between 15-20 minutes.

Note: *For other related products, please visit our web site at* ***www.GBiosciences.com***

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