Aalto University
School of Chemical
Engineering

Laboratory course in Biosystems and Biomaterials

Part I – Enzyme kinetics.

September 29th, 2023

Organizers and instructors:

Laura Niemelä (Molecular Biotechnology)

Rahul Mangayil (Biomolecular Materials)

Department of Bioproducts and Biosystems | Aalto University

Table of Contents

CHEM-E8110: Day 1 - Acid phosphatase assay 3

Introduction *3*

Materials 3

Procedure 4

CHEM-E8110: Day 1 - Acid phosphatase assay

Introduction

Saccharomyces cerevisiae produces acid phosphatase endogenously. As the produced phosphatase catalyzes dephosphorylation reactions the presence of the acid phosphatase can be tested/ quantified with a colorimetric assay based on the dephosphorylation reaction of para-nitrophenyl phosphate (pNPP) to para-nitrophenol (pNP) (figure 1).

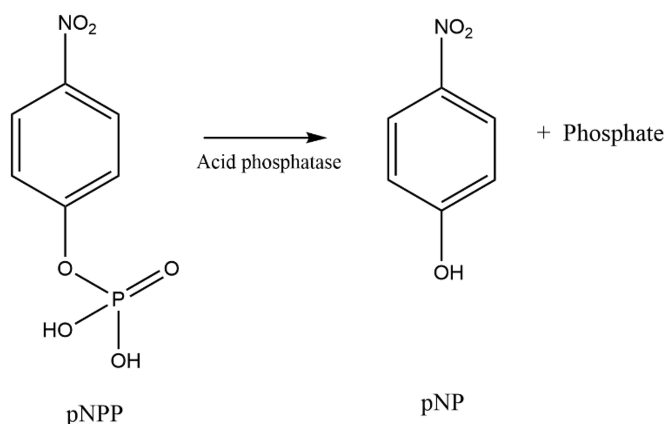


Figure 1. Acid phosphatase catalyzes the dephosphorylation of pNPP to pNP which produces a color reaction that can be quantified by reading the absorbance at the wavelength of 405 nm.

<u>Learning outcomes</u>: During this exercise you will learn basic pipetting skills and how to conduct a simple assay.

<u>Aim</u>: The aim of this assay is to quantify the produced acid phosphatase from the yeast strain that overexpresses the enzyme.

Materials

- Yeast culture samples (two students share one tube)
- Substrate (20 mM pNPP in buffer)
- Standard solution tube Nr.1 (50 mM pNP in buffer)
- 100 mM Na-acetate buffer pH 4.2 (for creating the dilution series of the standard)
- Stop solution (2M Na_2CO_3)
- Blank (culture media)
- Eppendorf tubes

Procedure

In a nutshell:

Task 1. Create a dilution series for the standard curve (in Eppendorf tubes).

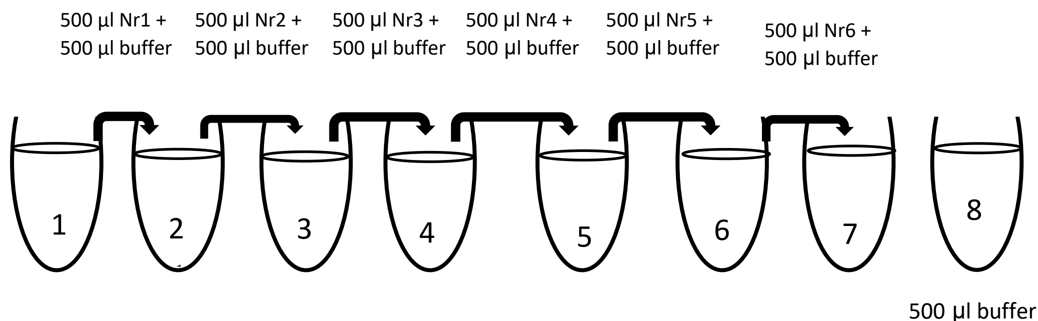
Task 2. Pipette the samples and blank in Eppendorf tubes for the assay.

Task 3. Conduct the assay: Add the substrate into the Eppendorf tubes containing the sample and blank and incubate the tubes at 30°C. Quench the reactions with the stop solution.

Task 4. Both the standard, sample and control reactions are transferred to 96 well plate for absorbance reading (done by teaching assistant)

<u>Procedure in detail</u>

1. Create a 1:2 dilution series of the pNP -standard solution (50 mM).
    a. Create seven dilutions and the number (8) of the dilution series contains only Na-acetate buffer.



2. Pipette 60 µl of sample in Eppendorf tubes which you mark as 10 min, 20 min and 30 min samples. Pipette 60 µl of media as blank in one tube.
3. Add 300 µl of 20 mM substrate in timed intervals (e.g. 10s) to the sample and blank tubes.
4. Incubate the tubes at 30°C (thermoblock). The reaction is stopped at timed intervals (10 min, 20 min, 30 min) with 600 µl stop solution (2M Na_2CO_3).
5. Now the dilution series of the standard you created, the samples and controls are pipetted in a 96 well plate (Done by assistant).
6. The absorbance of the color reaction can be read with a plate reader at a wavelength of 405 nm. (Done by teaching assistant)

<u>Questions</u>

- Prepare a standard curve from the data. What needs to be plotted?
- What is the pNPP turnover catalyzed by the yeast produced acid phosphatase?

- Calculate the turnover rate (substrate converted / time unit) of pNPP to pNP with the help of the standard curve.
    a. Report your answers in MyCourses.

	1	2	3	4	5	6	7	8	9	10	11	12	
A													A
B													B
C													C
D													D
E													E
F													F
G													G
H													H