Biology Lab. 2
– Enzyme assay –

	Student ID	Name
Experimenter		
Exp. partner		
Exp. period		

Result 1: Protein concentration in blood plasma

Determine the protein concentration in provided blood plasma by measuring the absorbance

Table 1: Absorbance of blood plasma

Wave length/nm	**280**
Absorbance	

This value is obtained from _________ times diluted sample of blood plasma.

Protein concentration can be expressed by the following equation;

$C = K \times A$

C: protein concentration in blood plasma

K: conversion factor

A; Absorbance of sample

[note] The conversion factor *K* is 1.15.

Thus, protein concentration in undiluted blood plasma is

__________________ mg/mL.

Result 2: Measurement of enzyme activity

Table 2: Measurement of enzyme activities using undiluted substrate and undiluted blood plasma

Time/min	Absorbance (A_{340})
0.5	
1	
1.5	
2	
2.5	
3	
3.5	
4	
4.5	
5	

Result 3: The effect of substrate concentration on enzyme activity

Based on the results of Table 2, blood plasma is diluted ______ times not to make the difference in absorbance within 1 min exceed 0.050.

Table 3: Changes in absorbance (A_{340}) at different concentrations of the substrate with constant blood plasma concentration

Time/min	Substrate concentration							
	Undiluted 1 mM	Conc-1	Conc-2	Conc-3	Conc-4	Conc-5	Conc-6	Conc-7
0.5								
1								
1.5								
2								
2.5								
3								
3.5								
4								
4.5								
5								

Based on the results of Table 3, make a graph with incubation time on transverse axis and absorbance (A_{340}) on vertical axis (Figure 1).

[note] Add trend line and show the equation to the graph.

Calculate the reaction rate (v) from absolute value of the slope of the curve (change in absorption per min) in each substrate concentration (Table 4).
The molar absorptivity of NADH at 340 nm is $6.22 \times 10^3\ M^{-1}cm^{-1}$ for a cuvette with an optical path length of 1 cm.

[note] M = mole/L

Table 4: Changes in absorbance and reaction rate in different substrate concentrations

Substrate conc.([S]) (mM)	**1**							
Change in absorption per min (min^{-1})								
Reaction rate (v) (μM/min)								

On the basis of these results in Table 4, make a graph (Figure 2) with substrate concentration ([S]) on transverse axis and the reaction rate (v) on vertical axis.
Also make a Lineweaver-Burk plot (Figure 3) by taking double-reciprocal values (1/[S] as a transverse axis and $1/v$ as vertical axis).

[note] Substrate concentration can be calculated with undiluted substrate concentration and the added volume to the reaction.
(example) two times dilution
$30\ mM \div 2 \times (0.1\ mL \div 3\ mL) = 0.5\ mM$

[note] Add the trend line to Figure 2. <u>Draw the trend line to the x-intercept</u> and add the equation to Figure 3.

Based on the equation in Figure 3, the values of *Km* and *Vmax* of enzyme in blood plasma can be calculated as follows;

Km = ____________________ mM

Vmax = ____________________ μM/min

Details of the calculation (You may add extra pages if needed.)

Result 4: The effect of blood plasma concentration on enzyme activity

Table 5: Changes in absorbance (A_{340}) at different concentrations of the blood plasma with constant substrate concentration

Time/ min	Un-diluted (mg/mL)	Dilution rate: 1/2	Dilution rate:	Dilution rate:	Dilution rate:	Dilution rate:	Dilution rate:
0.5							
1							
1.5							
2							
2.5							
3							
3.5							
4							
4.5							
5							

Based on Table 5, make a graph (Figure 4) with time on transverse axis and absorbance (A_{340}) on vertical axis.

[note] Add trend line and corresponding equation to Figure 4.

Calculate the reaction rate (v) from absolute value from the slopes of the equations in each substrate concentration (Table 6).

[note] Convert dilution rates into amounts of blood plasma (E).

Table 6: Changes in absorbance and reaction rate in different blood plasma concentrations

Dilution rate	1	1/2					
Amount of blood plasma (E) (mg)							
Change in A_{340} (min^{-1})							
Reaction rate (v) (μM/min)							

Based on Table 6, make a graph (Figure 5) with the amount of blood plasma (E) on transverse axis and the reaction rate (v) on vertical axis.

[note] The amount of blood plasma can be calculated with undiluted blood plasma concentration (from Result 1) and the added volume to the reaction.

(example) If you diluted 50 mg/mL two times,
$50 \text{ mg/mL} \div 2 \times 0.1 \text{ mL} = 2.5 \text{ mg}$

[note] Add the trend line and the equation to Figure 5.