Vitamin B1

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1175

(Version 1.3A)

Detection and Quantification of Vitamin B1(VB1)content in Urine, Tissue extracts, Cell lysate, Other biological fluidsSamples.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

Vitamin B1, also known as thiamin, is a vitamin found in food, and manufactured as a dietary supplement and medication. Food sources of thiamine include whole grains, legumes, and some meats and fish. Grain processing removes much of the thiamine content, so in many countries cereals and flours are enriched with thiamine. Supplements and medications are available to treat and prevent thiamine deficiency and disorders that result from it, including beriberi and Wernicke encephalopathy. Other uses include the treatment of maple syrup urine disease and Leigh syndrome. Vitamin B1Colorimetric Microplate Assay Kit is a sensitive assay for determining Vitamin B1content in various samples.Vitamin B1content is determined by the prussian blue reaction. The increase in absorbance at 704 nm is directly proportional to the content.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Substrate	Powderx 1	4 °C, keep in dark
SubstrateDiluent	5 ml x 1	4 °C
Reaction Buffer	7 ml x 1	4 °C
Dye Reagent	Powderx 1	4 °C, keep in dark
Dye Reagent Diluent	3 ml x 1	4 °C
Standard	Powderx 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 704 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Hot air circulating oven

IV. REAGENT PREPARATION

Substrate: Add 5 ml SubstrateDiluent to dissolve before use.Keep in dark and store at 4 °C for 2-3 weeksafter reconstitution.

Dye Reagent: Add 3 ml Dye Reagent Diluent to dissolve before use.Keep in dark and store at 4 °C for 3 monthsafter reconstitution.

Standard:Briefly centrifuge prior to opening.Dissolvein1 ml distilled waterto generate 10 mmol/Lstandardstock solution.Then dilute to200 µmol/L standard top solutionby adding 20 µl stock solution into 980µl distilled water. Perform 2-fold serial dilutions of the top standardsolution using distilled waterto make the standard curve.The concentration of standard curve could be200/100/50/25/12.5/6.25/3.12µmol/L. The standards should be use within 24 hours.

V. SAMPLE PREPARATION

1.For urine andother biological fluids samples

Detect directly.

2.For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml0.05 mol/L H_2SO_4 (not provided), put it in boiling water for 15 minutes; when cold, add 2.5 mol/L NaAc (not provided) and adjust the pH to 4.5, then add glucoamylase (not provided), keep it at 55°C overnight; thencentrifuged at 10000g for 10minutes, take the supernatant into a new centrifuge tube for detection.

VI. ASSAY PROCEDURE

Add following reagents intothe microplate:

Reagent*	Sample**	Standard	Blank
Sample	50 µl	--	--
Standard	--	50 µl	
Distilled water	--	--	50 µl
Substrate	50 µl	50 µl	50 µl
Mix, put it into the hot air circulating oven, 80°C for 10 minutes, then put it on ice.			
Reaction Buffer	70 µl	70 µl	70 µl
Dye Reagent	30 µl	30 µl	30 µl
Mix, incubate at room temperature for 10 minutes, record absorbance measured at 704nm.			

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

1. Calculatethe sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

$$C=\frac{(OD_{Sample}-OD_{Blank})-\text{Intercept}}{\text{Slope}}\times n(\mu mol/L)$$

Calculate the initial concentration according to sample preparation procedure.

2. According to one point of the standard OD and concentration

2.1 According to the weight of sample

$$C=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank})\times W\times(V_{Sample}/V_{Assay})}(\mu mol/g)$$

2.2According to the volume of sample

$$C=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank})\times V_{Sample}}(\mu mol/ml)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

$C_{Standard}$: the standard concentration, mmol/L = μmol/ml

$V_{Standard}$: the volume of standard in assay procedure, μl

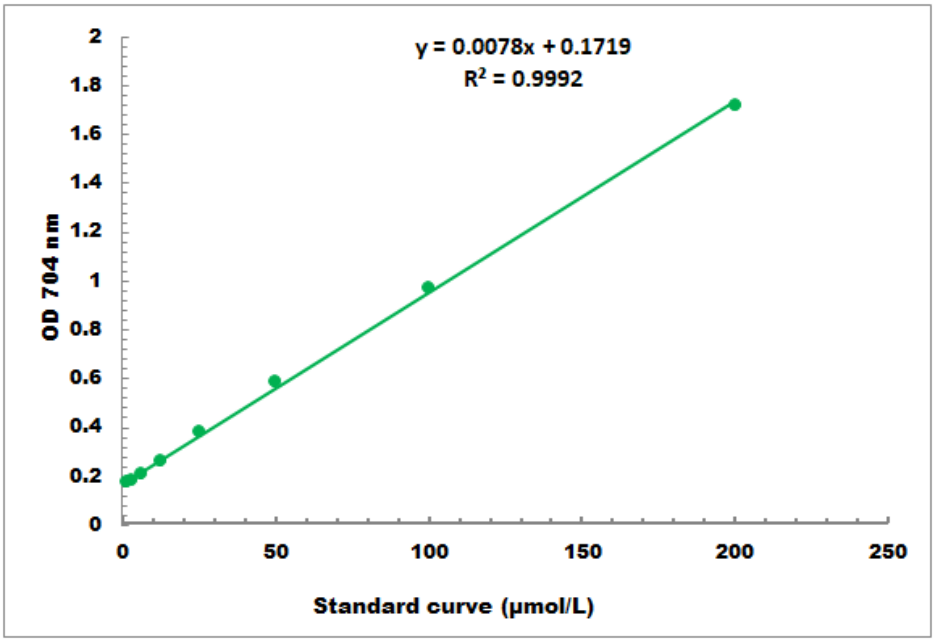
V_{Sample}: the volume of sample in assay procedure, μl

V_{Assay}: the volume of Assay Buffer, μl

W: the weight of sample, g

VIII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 2 µmol/L - 200 µmol/L