



# Vitamin B1 Microplate Assay Kit

## User Manual

Catalog # ASK1175

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

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

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I. INTRODUCTION.....2

II. KIT COMPONENTS.....3

III. MATERIALS REQUIRED BUT NOT PROVIDED.....3

IV. SAMPLE PREPARATION.....4

V. ASSAY PROCEDURE.....5

VI. CALCULATION.....6

VII. TYPICAL DATA.....7



**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 B1 Microplate Assay Kit is a sensitive assay for determining Vitamin B1 content in various samples. Vitamin B1 content 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	Powder x 1	4 °C
Substrate Diluent	5 ml x 1	4 °C
Reaction Buffer	7 ml x 1	4 °C
Dye Reagent	Powder x 1	4 °C
Dye Reagent Diluent	3 ml x 1	4 °C
Standard	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

**Note:**

Substrate: add 5 ml Substrate Diluent to dissolve before use.

Dye Reagent: add 3 ml Dye Reagent Diluent to dissolve before use.

Standard: add 1 ml distilled water to dissolve before use; then add 20 µl into 980 µl distilled water, mix, the concentration will be 200 µmol/L.

**III. MATERIALS REQUIRED BUT NOT PROVIDED**

1. Microplate reader to read absorbance at 704 nm
2. Distilled water
3. Pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer



**IV. SAMPLE PREPARATION**

1. For urine and other biological fluids samples

Detect directly.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml 0.05 mol/L H<sub>2</sub>SO<sub>4</sub> (not provided), put it in boiling water for 15 minutes; when old, 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; then centrifuged at 10000g for 10 minutes, take the supernatant into a new centrifuge tube for detection.



**V. ASSAY PROCEDURE**

Add following reagents into the microplate:

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

**VI. CALCULATION**

1. According to the volume of sample

$$\begin{aligned} \text{VB1 } (\mu\text{mol/ml}) &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / \\ &V_{\text{Sample}} \\ &= 0.2 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \end{aligned}$$

2. According to the protein concentration of sample

$$\begin{aligned} \text{VB1 } (\mu\text{mol/mg}) &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / \\ &(V_{\text{Sample}} \times C_{\text{Protein}}) \\ &= 0.2 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / C_{\text{Protein}} \end{aligned}$$

3. According to the weight of sample

$$\begin{aligned} \text{VB1 } (\mu\text{mol/g}) &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (V_{\text{Sample}} \\ &\times W / V_{\text{Assay}}) \\ &= 0.2 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / W \end{aligned}$$

$C_{\text{Standard}}$ : the standard concentration, 200  $\mu\text{mol/L}$  = 0.2  $\mu\text{mol/ml}$ ;

$C_{\text{Protein}}$ : the protein concentration, mg/ml;

W: the weight of sample, g;

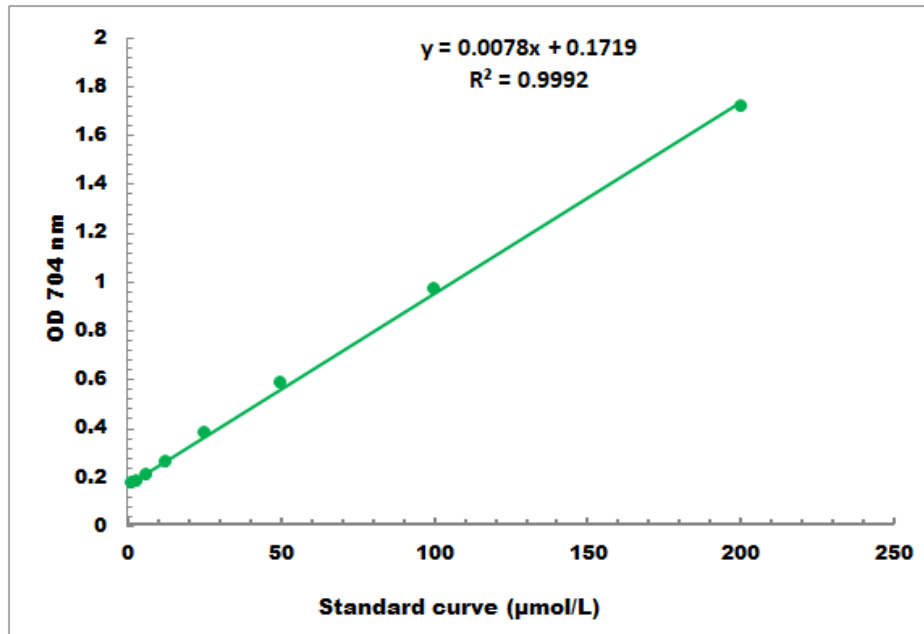
$V_{\text{Standard}}$ : the volume of standard, 50  $\mu\text{l}$ ;

$V_{\text{Sample}}$ : the volume of sample, 50  $\mu\text{l}$ ;

$V_{\text{Assay}}$ : the volume of Assay buffer, 1 ml;

**VII. 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