Vitamin B1 Microplate Assay Kit

User Manual

Catalog # FTA0158

(Version 1.2A)

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.

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, multi-channel 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_2SO_4 (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 µl	--	--
Standard	--	50 µl	
Distilled water	--	--	50 µl
Substrate	50 µl	50 µl	50 µl
Mix, put it into the 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:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) 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.

VI. CALCULATION

1. According to the volume of sample

$$VB1\ (\mu mol/ml) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / V_{Sample}$$

$$= 0.2 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$$

2. According to the protein concentration of sample

$$VB1\ (\mu mol/mg) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein})$$

$$= 0.2 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

3. According to the weight of sample

$$VB1\ (\mu mol/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times W / V_{Assay})$$

$$= 0.2 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

$C_{Standard}$: the standard concentration, 200 μmol/L = 0.2 μmol/ml;

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

W: the weight of sample, g;

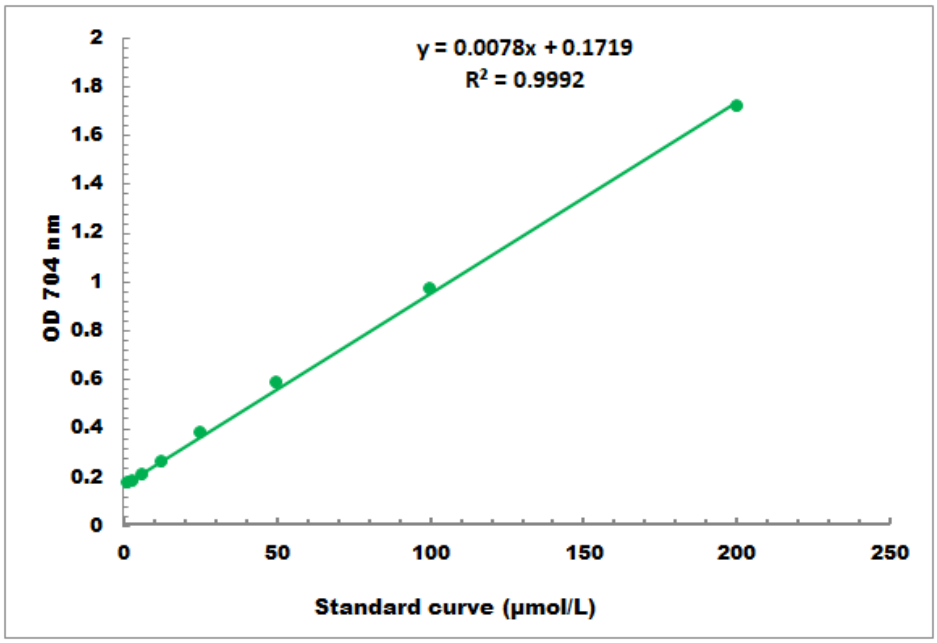
$V_{Standard}$: the volume of standard, 50 μl;

V_{Sample}: the volume of sample, 50 μl;

V_{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

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES