Vitamin B6 Microplate Assay Kit

User Manual

Catalog # CAK1176

(Version 1.3A)

Detection and Quantification of Vitamin B6 (VB6) content in Tissue extracts, Other biological fluids Samples.

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

I. INTRODUCTION

Vitamin B6, also known as pyridoxine, which includes pyridoxine, pyridoxal and pyridoxamine, is present in the form of phosphate in the body. Vitamin B6 is a colorless crystal, soluble in water and ethanol, stable in acid solution, easily destroyed in alkali solution, pyridoxine heat, pyridoxal and pyridoxamine are not resistant to high temperature. Vitamin B6 is abundant in yeast, liver, grain, meat, fish, eggs, beans and peanuts. Vitamin B6 is a component of certain coenzymes in the human body and is involved in a variety of metabolic reactions, especially in relation to amino acid metabolism.

Vitamin B6 Microplate Assay Kit is a sensitive assay for determining Vitamin B6 content in various samples. Vitamin B6 content is determined by the aminoantipyrine. The increase in absorbance at 390 nm is directly proportional to the content.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Substrate	Powder x 1	4 °C
Reaction Buffer	5 ml x 1	4 °C
Dye Reagent	Powder x 1	4 °C
Standard	Powder x 1	4 °C
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Note:

Substrate: add 5 ml distilled water to dissolve before use.

Dye Reagent: add 8 ml distilled water to dissolve before use.

Standard: add 1 ml distilled water to dissolve before use, then add 0.25 ml into 0.75ml distilled water, mix; the concentration will be 5 mmol/L.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 390 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer

IV. SAMPLE PREPARATION

1. For tissue extracts, other biological fluids samples

Detect directly.

V. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent	Sample	Standard	Blank
Sample	20 µl	--	--
Standard	--	20 µl	--
Distilled water	--	--	20 µl
Reaction Buffer	50 µl	50 µl	50 µl
Dye Reagent	80 µl	80 µl	80 µl
Substrate	50 µl	50 µl	50 µl
Mix, incubate at room temperature for 20 minutes, record absorbance measured at 390nm.			

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.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

1. According to the volume of sample

$$VB6\ (mmol/L) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / V_{Sample}$$

$$= 5 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$$

2. According to the protein concentration of sample

$$VB6\ (mmol/mg) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein})$$

$$= 5 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

$C_{Standard}$: the standard concentration, 5 mmol/L;

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

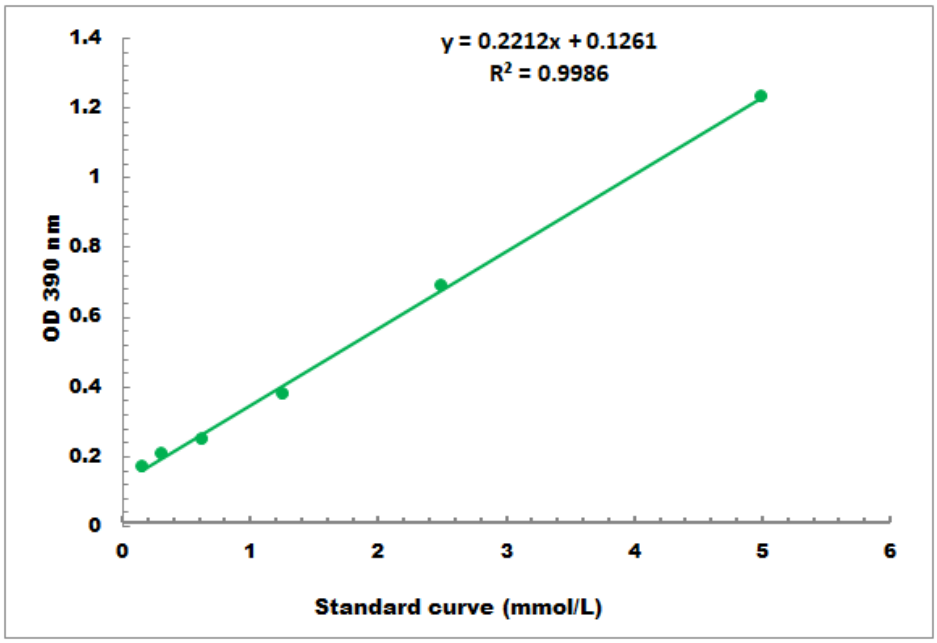
W: the weight of sample, g;

$V_{Standard}$: the volume of standard, 20 µl;

V_{Sample}: the volume of sample, 20 µl.

VII. TYPICAL DATA

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



Detection Range: 0.1 mmol/L - 5 mmol/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