Pyridoxine Hydrochloride / Vitamin B6 Assay Kit

ARG83393 Pyridoxine Hydrochloride / Vitamin B6 Assay Kit can be used to measure Pyridoxine Hydrochloride / Vitamin B6 in Tissue extracts and other biological fluids.

Catalog number: ARG83393

Package: 96 wells

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INTRODUCTION

Pyridoxine Hydrochloride is the hydrochloride salt form of pyridoxine, a water-soluble vitamin B. Pyridoxine hydrochloride is converted into the active form, pyridoxal 5'-phosphate (PLP), an essential cofactor in many enzymatic activities including synthesis of amino acids, neurotransmitters, and sphingolipids. This vitamin is essential to red blood cell, nervous system, and immune systems functions and helps maintain normal blood glucose levels.

PRINCIPLE OF THE ASSAY

The ARG83393 Pyridoxine Hydrochloride / Vitamin B6 Assay Kit determined Vitamin B6 by the aminoantipyrine. The increase in absorbance at 390 nm is directly proportional to the content.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8°C. Use the kit before expiration date.

Component	Quantity	Storage
Microplate	1 X 96-well plate	
Standard	1 vial (lyophilized)	4°C
Substrate	1 vial (lyophilized)	4°C
Reaction Buffer	5 ml	4°C
Reaction Dye	1 vial (lyophilized)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 390 nm
- Pipettes and pipette tips
- Deionized or distilled water

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store all component at 4°C.
- Reaction Dye should be store at 4°C and protect from light.
- Briefly spin down the reagents before use.
- It is highly recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Note: For other liquid sample, it can be assayed directly.

REAGENT PREPARATION

- **Standard:** Add **1 ml** of **distilled water** to dissolve before use (to yield 20 µmol/ml as stock); then add 250 µl into 750 µl distilled water. The concentration will be 5 µmol/ml. Perform 2-fold serial dilution of the top standards to make the standard curve.
- **Reaction Dye**: Reconstitute the Substrate with **8 ml** of **distilled water**. Allow the Reaction Dye keep on bench for few minutes. Make sure the Reaction Dye is dissolved completely and mixed thoroughly before use. Keep the reconstituted the Substrate on ice before use.
- **Substrate:** Reconstitute the Substrate with **5 ml** of **Substrate Diluent**. Allow the Substrate keep on bench for few minutes. Make sure the Substrate is dissolved completely and mixed thoroughly before use. Keep the reconstituted the Substrate on ice before use. Keep the reconstituted the Substrate on ice before use.
- **Sample**: If the measuring absorbance of samples is higher than the standard, dilute the samples with **distilled water** before assay and assay again. For the calculation of the activity this dilution factor has to be taken into account.

ASSAY PROCEDURE

Standards and samples should be assayed in at least duplicates.

1. Sample wells: Add **20 µl** per **samples** into each microplate.
2. Standard wells: Add **20 µl** of **Standard** into microplate.
3. Add **50 µl Reaction Buffer** to All wells.
4. Add **80 µl** of **Reaction Dye** per well into All wells.
5. Add **50 µl Substrate** to All wells.
6. Mix well. Incubate at **RT** for **20 min**.
7. Read the OD with a microplate reader at **390 nm**.

Summary of Pyridoxine Hydrochloride / Vitamin B6 Assay Procedure

Reagent	Sample	Standard	Blank
Sample	20 µl	-	-
Standard	-	20 µl	-
Distilled water	-	-	20 µl
Reaction Buffer	50 µl	50 µl	50 µl
Reaction Dye	80 µl	80 µl	80 µl
Substrate	50 µl	50 µl	50 µl
Mix well. Incubate at **RT** oven for **20 min**.			
Read the OD with a microplate reader at **390 nm**.			

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of samples, standard, positive control, control and blank.

2. Calculation:

A. Definition:

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

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

W: the weight of sample, g;

V_{Sample}: the volume of reaction sample, 20 µl = 0.02 ml;

$V_{standard}$: the volume of standard sample, 20 µl = 0.02 ml;

B. Formula:

a). According to the volume of sample

Pyridoxine Hydrochloride / Vitamin B6 (mmol /ml) =

$[(C_{Standard} \times V_{standard}) \times (OD_{Sample} - OD_{Blank})] / [(OD_{Standard} - OD_{Blank}) \times V_{Sample}]$

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

b). According to the concentration of sample

Pyridoxine Hydrochloride / Vitamin B6 (mmo/mg) =

$[(C_{Standard} \times V_{standard}) \times (OD_{Sample} - OD_{Blank})] / [(OD_{Standard} - OD_{Blank}) \times (V_{Sample} \times C_{Protein})]$

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

3 Detection range:

The detection range is from 0.1 mmol/ml – 5 mmol/ml.

4. If the samples have been diluted, the calculated concentration must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL RESULT

The following data is for demonstration only and cannot be used in place of data generations at the time of assay. Please note this data is for demonstration only and serial diluted standards are not necessary for this kit.