Thiamine / Vitamin B1 Assay Kit

ARG83392 Thiamine / Vitamin B1 Assay Kit can be used to measure Thiamine / Vitamin B1 in Urine, tissue extracts, cell lysate and other biological fluids.

Catalog number: ARG83392

Package: 96 wells

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MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: No. 22, Ln. 227, Gongyuan Rd., Hsinchu City 300, Taiwan

Phone: +886 (3) 562 1738

Fax: +886 (3) 561 3008

Email: info@arigobio.com

INTRODUCTION

Thiamine or thiamin, also known as vitamin B1, is a colorless compound with the chemical formula C12H17N4OS. It is soluble in water and insoluble in alcohol. Thiamine decomposes if heated. Thiamine was first discovered by Umetaro Suzuki in Japan when researching how rice bran cured patients of Beriberi. Thiamine plays a key role in intracellular glucose metabolism and it is thought that thiamine inhibits the effect of glucose and insulin on arterial smooth muscle cell proliferation. Thiamine plays an important role in helping the body convert carbohydrates and fat into energy. It is essential for normal growth and development and helps to maintain proper functioning of the heart and the nervous and digestive systems. Thiamine cannot be stored in the body; however, once absorbed, the vitamin is concentrated in muscle tissue.

PRINCIPLE OF THE ASSAY

The ARG83392 Thiamine / Vitamin B1 Assay Kit determine Vitamin B1 by the prussian blue reaction. The increase in absorbance at 704 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
Assay Buffer	4 X 30 ml (ready to use)	4°C
Substrate	1 vial (lyophilized)	4°C
Substrate Diluent	5 ml	4°C
Reaction Dye	1 vial (lyophilized)	4°C
Dye Diluent	3 ml	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 704 nm
- Pipettes and pipette tips
- Deionized or distilled water
- Convection oven (80°C)

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.

Tissue lysate- Weigh out 0.1 g of tissue, homogenize with 0.05 mol/L H_2SO_4, 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.

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 10 mmol/ml as stock); then add 20 µl into 980 µl distilled water. The concentration will be 200 µmol/ml. Perform 2-fold serial dilution of the top standards to make the standard curve.
- Reaction Dye: Reconstitute the Substrate with 3 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 **1X Assay buffer** 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 **50 µl** per **samples** into each microplate.
2. Standard wells: Add **50 µl** of **Standard** into microplate.
3. Add **50 µl Substrate** to All wells.
4. Mix well and incubate all wells at **80°C** oven for **10 min**, put it **on ice**.
5. Add **70 µl** of **Reaction Buffer** per well into all wells.
6. Add **30 µl** of **Reaction Dye** per well into all wells.
7. Mix well. Incubate at **RT** for **10 min**.
8. Read the OD with a microplate reader at **704 nm**.

Reagent	Sample	Standard	Blank
Sample	50 µl	-	-
Standard	-	50 µl	-
Distilled water	-	-	50 µl
Substrate	50 µl	50 µl	50 µl
Mix well. Incubate all Sample tubes at **80°C** oven for **10 min**.			
Reaction Buffer	70 µl	70 µl	70 µl
Reaction Dye	30 µl	30 µl	30 µl
Mix well. Incubate at **RT** oven for **10 min**.			
Read the OD with a microplate reader at **704 nm**.			

CALCULATION OF RESULTS

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

3. Calculation:

A. Definition:

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

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

W: the weight of sample, g;

V_{Sample}: the volume of reaction sample, 50 µl = 0.05 ml;

$V_{standard}$: the volume of standard sample, 50 µl = 0.05 ml;

V_{assay}: the volume of assay buffer, 1000 µl = 1 ml;

B. Formula:

a). According to the volume of sample

Thiamine / Vitamin B1 (µmol /ml) =

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

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

b). According to the concentration of sample

Thiamine / Vitamin B1 (µmol /mg) =

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

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

c). According to the quantity of weight or sample

Thiamine / Vitamin B1 (μmol /g) =

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

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

4. Detection range:

The detection range is from 0.2 μmol/ml- 200 μmol/ml.

5. If the samples have been diluted, the calculated activity 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.