VB6 Enzymatic

Vitamin B6 Enzymatic Assay

This product is for research use only
It is not intended for use in diagnostic procedures

KK-VB6-U 100 tests

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ENGLISH

INTENDED USE

The BÜHLMANN Vitamin B6 enzymatic assay (KK-VB6) is intended for the quantitative determination of Pyridoxal 5'-Phosphate (PLP, vitamin B6) in EDTA plasma.
This product is for research use only. It is not intended for use in diagnostic procedures

PRINCIPLE OF THE ASSAY

L-Tyrosine is decarboxylated by a vitamin B6 (PLP)-dependent enzyme, tyrosine-apo-decarboxylase to tyramine. The activity of the apo-enzyme is directly proportional to the amount of PLP present in the reaction mixture. Tyramine is then oxidized to p-hydroxybenzyl aldehyde and hydrogen peroxide (H_2O_2) by the action of tyramine oxidase. The H_2O_2 reacts with 4-aminoantipyrine and N-ethyl-N-(2-hydroxy-3-sulfopropyl)-m-toluidine (TOOS) in the presence of horseradish peroxidase to obtain a quinoneimine (purple dye) whose absorbance is measured at 546 nm (520-595 nm).

REAGENTS SUPPLIED AND PREPARATION

Reagents[1)]		Quantity	Code	Reconstitution
Dilution Buffer		1 vial 60 ml	B-KVB6-DB	Ready to use
Enzyme Buffer		1 vial 13 ml	B-KVB6-EB	Ready to use
Substrate	R1	1 vial lyophilized	B-KVB6-SUB	Add 5 ml of Dilution Buffer
Apo-Enzyme	R2	1 vial lyophilized	B-KVB6-APOE	Add 5 ml of Dilution Buffer
Enzyme	R3	2x 1 vial lyophilized	B-KVB6-E	Add 5 ml of Enzyme Buffer
Calibrators[2]		3x 1 vial lyophilized	B-KVB6-CASET	Add 2 ml of Dilution Buffer
Controls Low and Normal		2x 1 vial lyophilized	B-KVB6-CONSET	Add 2 ml of Dilution Buffer

Table 1

1) Reconstitute the lyophilized reagents as indicated in chapter Procedural Notes.

2) After reconstitution the PLP concentration of the Calibrators is **0 (Blank), 20, and 200 nmol/L**. The samples must be diluted 1:40 for the measurement in the enzyme assay. The indicated concentrations of the reconstituted calibrators are taking this dilution factor into account and contain the following effective concentrations: 0, 0.5, and 5 nmol/L.

STORAGE AND SHELF LIFE OF REAGENTS

Unopened Reagents	
All reagents are stable at 2-8°C until expiration date	
Opened / Reconstituted Reagents	
Substrate	stable for 2 months **at 2-8 °C**
Dilution Buffer	
Enzyme Buffer	
Enzyme	
Apo-Enzyme	**Stable at ≤-20 °C for 2 months; Store in aliquots, if reagent is needed for more than 3 runs.**
Calibrators[2]	
Controls Low and Normal	

Table 2

WARNINGS AND PRECAUTIONS

Plasma samples may be potentially infectious and should be handled according to good laboratory practice using appropriate precautions.

MATERIALS REQUIRED BUT NOT PROVIDED

- 25 µl, 50 µl, 500 µl, 1000 µl precision pipettes and multipipette with disposable tips for 50 µl and 100 µl.
- 5 ml volumetric pipette
- Disposable 2 ml polypropylene screw-cap microtube
- Microtiter plate MaxiSorp F8 (NUNC, Code: 468667) or equivalent
- Microtiter plate reader with filter between 520 and 595 nm (maximum absorbance at 546 nm)
- Microtiter plate incubator set at 37 °C
- Microtiter plate shaker

SPECIMEN COLLECTION AND STORAGE

EDTA plasma samples

Minimum volume of 0.5 ml of blood is recommended for duplicate determination. Draw blood into an EDTA venipuncture tube. Centrifuge for 15 minutes at 1000 x g at 2-8 °C immediately after collection or after storage at 2-8 °C for up to 12 hours protected from light. **Avoid long exposure to light.** After centrifugation, collect the plasma in polypropylene tubes and store at ≤ -20 °C if not assayed immediately. PLP in plasma will remain stable for at least 3 months if stored at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

LIMITATIONS

Avoid lipemic and hemolytic plasma.
Lipemic plasma: Samples should be taken from fasting individuals because interferences occur in the photometric determination otherwise.
Hemolytic plasma: Slightly hemolytic samples can be used. Refer to Table 14.
Specimens other than EDTA plasma have not been validated.
Apo-Enzyme, Calibrators, and Controls must be stored at -20 °C after reconstitution. They are stable at ≤-20 °C for 2 months; Store in aliquots, if reagent is needed for more than 3 runs.

PROCEDURAL NOTES

Reconstitution of reagents

Let the dilution buffer adjust to reach **room temperature**. Reconstitute the lyophilized reagents as indicated, vortex the vials for 30 seconds and leave them for at least 15 minutes at room temperature or use a suspension mixer for 15 minutes. Mix well (vortex) the reagents before use. **Important:** The Apo-Enzyme reagent (B-KVB6-APOE) must be constantly mixed until the lyophilized enzyme has completely dissolved.

ASSAY PROCEDURE

Preparation of samples and controls

Samples and controls have to be diluted 1:40 with dilution buffer. **Diluted samples and controls are not stable. Thus prepare dilution immediately before usage.** E.g. pipet 25 µl of sample or control into a disposable polypropylene microtube, add 975 µl of Dilution Buffer and mix well (vortex).

In order to avoid temperature effects within the microtiter plate, leave the first and last strip of the test and the first and last well of each strip empty.

The test should be carried out **in duplicates**.

1. Pipet 50 µl of Substrate into wells of microtiter plate.
2. Pipet 50 µl of
   a. Calibrator 0 nmol/L (Blank)
   b. Calibrator 20 nmol/L
   c. Calibrator 200 nmol/L

d. Control Low (diluted)
e. Control Normal (diluted)
f. diluted samples

into the wells of microtiter plate.

3. Add 50 µl of Apo-Enzyme to each well.
4. Mix shortly (10-15 seconds) with a microtiter plate shaker.
5. Incubate the microtiter plate for 30 minutes at 37 °C (+ 5 minutes) in a plate incubator.
6. Pipet 100 µl of Enzyme with a multipipette (with disposable tips) into wells of microtiter plate.
7. Shake the plate gently (5-10 seconds) with a microtiter plate shaker.
8. Incubate the microtiter plate for 15 minutes at 37 °C (+3 minutes) in a plate incubator.
9. Read the OD at 546 nm (or at 520-595 nm) in a microtiter plate reader within 3-5 minutes.

CALCULATION OF RESULTS

Calibration curve

Use endpoint mode with two calibrators (20 and 200 nmol/L). Calibrator 0 is used as Blank. Read absorbances (OD) for Calibrator 0 (Blank), calibrators, controls and samples. Have the duplicates averaged for each calibrator, control, and sample and subtract the average Blank. Have a standard curve created by using linear curve-fitting. Refer to the instrument manual for further details.

Assay range: 9 - 250 nmol/L.

Calculation of results

If samples have been diluted higher than 1:40, the additional dilution factor must be factored in. Samples exceeding 250 nmol/L can be diluted with NaCl 0.9% or dilution buffer e.g. 1:3 and assayed again according to the assay procedure. The respective dilution factor must be factored in the calculation of results.

Refer to Table 3 and Figure 1 for typical results and standard curve. *These results and standard curve are provided for demonstration purposes only. A standard curve must be generated for each set of samples to be assayed.*

Conversion factor: nmol/L = ng/mL x 4.046

STANDARDIZATION

The VB6 Calibrators have been calibrated with UV/VIS spectrometry using the molar absorbance coefficient $\varepsilon_{389\ nm}$: 6666.7 $M^{-1}\,cm^{-1}$ (substrate: PLP in 0.1 N NaOH).

QUALITY CONTROL

The values of the Low and Normal Controls provided with the kit must be within the lot specific range indicated on the corresponding QC data sheet. Otherwise, the assay has to be repeated.

It is good laboratory practice (GLP) to record the following data for each assay: kit lot number, reconstitution dates of kit components, concentration value of controls, concentration values of internal pool sample.

INDICATORS OF DETERIORATION

A yellow coloration of the reconstituted Substrate reagent will not influence performance.

Visible signs of microbial growth and gross turbidity in the reagents may indicate degradation and warrant discontinuation of use.

PERFORMANCE CHARACTERISTICS

Limit of Blank (LoB): < 7 nmol/L. The LoB has been established by repeated measurements of blank values (0.9% NaCl, 1:40 in dilution buffer, n= 60) in accordance with CLSI protocol EP17-A.

Limit of Detection (LoD): < 7 nmol/L. The LoD has been established by repeated measurements of two samples containing 6 and 12 nmol/L PLP (n= 60) in accordance with CLSI protocol EP17-A.

Limit of Quantification (LoQ): Lower LoQ: ≤ 10 nmol/L. The LoQ was determined by repeated measurements of samples at concentrations between 6.3 and 298 nmol/L (n= 40). A limit of 10 % CV was applied.

Precision: Repeatability: <10 % CV; Total precision: <15 % CV. The precision has been determined in accordance with CLSI protocol EP5-A2 by repeated measurements in 2 runs per day over a period of 20 work days (Table 4).

Linearity: 9.0 - 250 nmol/L. Two samples with elevated PLP concentration have been diluted with low PLP samples in accordance with CLSI protocol EP6-A (refer to Figure 3).

Recovery: 81 - 105 %. Three samples have been spiked with increasing amounts of PLP and analyzed in 3 runs (Table 5).

Specificity: The substances listed in Table 6 have been analyzed between 30 and 10'000 nmol/L alone or in combination with 40 nmol/L PLP in order to determine the enzyme specificity.

INTERFERING SUBSTANCES

Interference substances were evaluated in accordance with CLSI protocol EP7-A2 by measurement of two samples.

No interference is detected with the following substances up to the listed concentrations: **triglycerides** (**Intralipid**® 200 mg/dL; equivalent to 5.6 mmol/L triglycerides), **conjugated bilirubin** (360 µmol/L; 30 mg/dL), **unconjugated bilirubin** (214 µmol/L; 12.5 mg/dL) or **haemoglobin** (3.2 mmol/L; 500 mg/dL).

Other substances and/or factors have not been investigated in this study. Interferences cannot be excluded.

CORRELATION

EDTA-plasma samples from subjects tested for vitamin B6 deficiency were used for correlation between HPLC and the two BÜHLMANN assays, the Radio-Enzymatic (RK-VB6) and the Enzymatic Assays (KK-VB6). The following correlations were established (refer to Figure 4 and Table 7).

APPENDIX I

TABLES

Figure 1: **Example of Standard Curve**

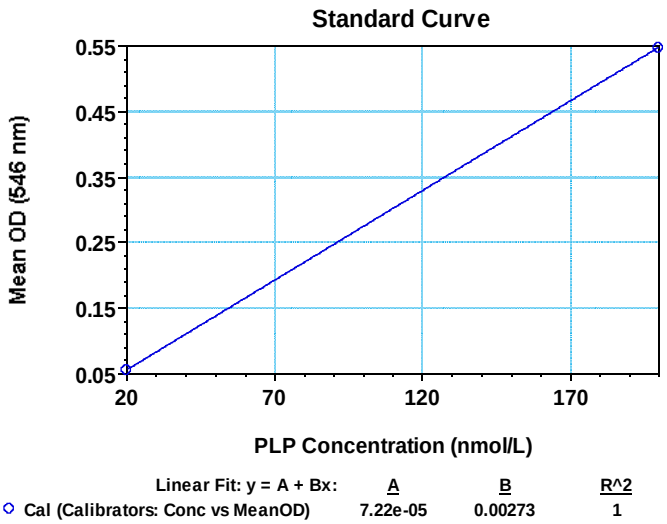

Table 3: **Example of Results**

Calibrator [nmol/L]	Netto OD*	Mean OD	CV (%)
20	0.054 0.057	0.056	3.8
200	0.556 0.561	0.559	0.7

OD_{brutto} Blank (Calibrator 0) = 0.183

Sample	Netto OD*	Result nmol/L	Mean Result nmol/L	CV (%)
Con low	0.0987 0.1067	35.3 38.2	36.8	5.5
Con normal	0.2696 0.2897	96.5 103.7	100.1	5.1
S1	0.0523 0.0463	18.7 16.6	17.6	8.6
S2	0.0657 0.0698	24.0 25.5	24.7	4.2
S3	0.1128 0.1130	39.8 39.9	39.9	0.1
S4	0.2934 0.3033	104.3 107.8	106.0	2.4
S5	0.4964 0.5019	176.7 178.6	177.6	0.8

*** Netto OD: Brutto OD - Blank (Calibrator 0).**

Table 4: **Precision (% CV)**

Sample	nmol/L	Repeatability (Within Run)	Between Run	Between Day	Total
Con Low	34.6	3.5%	9.1%	0.0%	9.8%
Con Normal	99.3	2.9%	6.2%	0.0%	6.8%
S1	15.7	6.0%	10.1%	0.0%	11.8%
S2	25.6	5.1%	7.2%	4.9%	10.0%
S3	39.5	2.7%	9.7%	0.0%	10.1%
S4	106.7	1.9%	5.2%	1.4%	5.7%
S5	170.6	1.6%	5.1%	0.0%	5.4%

Table 5: **Recovery**

Sample spiked with [nmol/L]	S1		S2	
	Obs [nmol/L]	O/E [%]	Obs [nmol/L]	O/E [%]
200	202.7	100	203.4	90
180	184.3	100	206.3	99
120	129.5	104	152.3	104
60	66.5	103	91.7	105
40	46.2	104	69.8	104
30	36.2	105	59.7	105
22.5	26.4	98	49.9	101
13.5	16.3	91	40.6	100
9.0	13.5	100	35.9	100
7.0	11.1	96	35.2	104
4.5	7.2	81	31.4	100
0	4.5	100	27.0	90
Mean		**98.5±6.75 %**		**100.2±5.2%**

Table 6: **Specificity of Tyrosine-apo-decarboxylase**

Component	Maximal tested concentration [nmol/L]	Interaction
Pyridoxal (PL)	< 10'000	≤ 0.1%
Pyridoxine (PN)		
Pyridoxamine (PM)		
4-pyridoxic acid (PA)		
Pyridoxamine 5'-phosphate (PMP)	< 1'200	≤ 0.2%
	< 10'000	≤ 0.8%

Figure 2: **Precision profile for EDTA plasma**

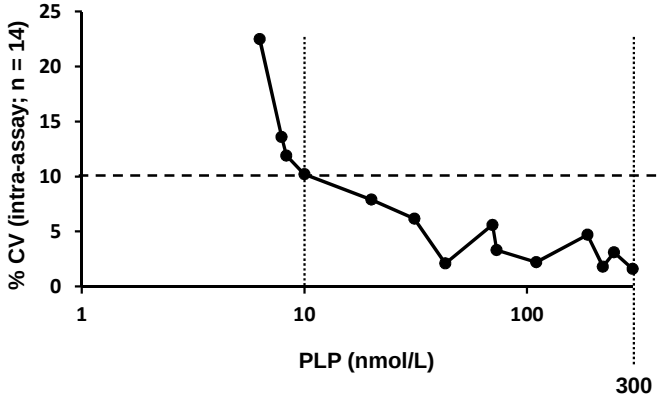

Figure 3 **Linearity**

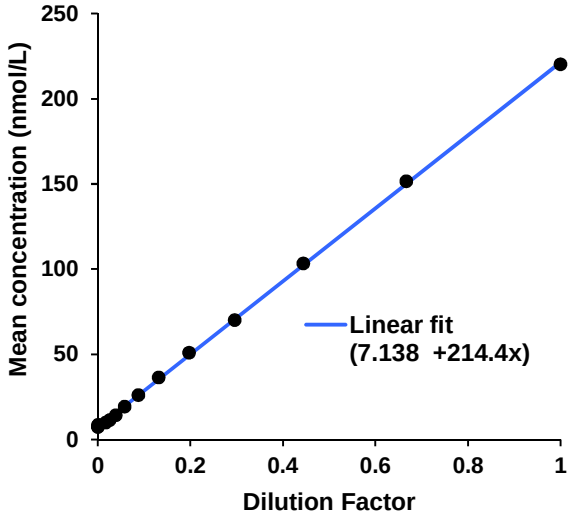

Figure 4 **Correlation with Vitamin B6 HPLC assay**

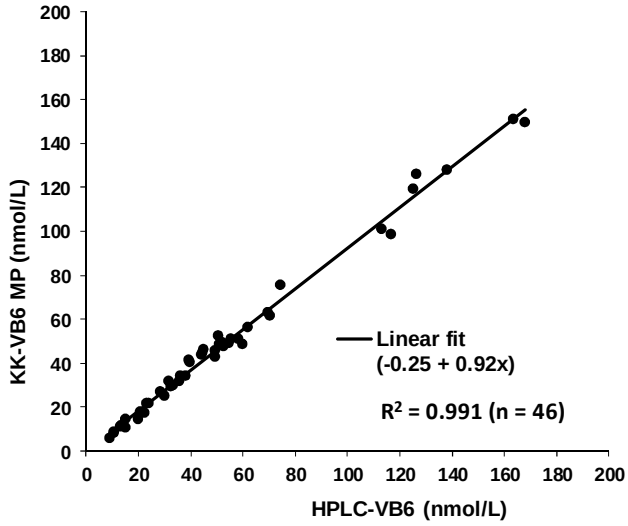

Table 7: **Correlation**

Correlation	n	R^2	Bias	Slope
KK-VB6 vs. HPLC	44	0.99	-0.06	0.92
KK-VB6 vs. RK-VB6	41	0.95	4.35	0.90
RK-VB6 vs. HPLC	44	0.97	-7.92	1.11

RK-VB6: BÜHLMANN Vitamin B6 Radio-Enzymatic Assay (REA)
HPLC: High-Performance Liquid Chromatography.

Table description: "Calculation of Results" (pg. 3), "Performance Characteristics" (pg. 3).

APPENDIX II
REFERENCES

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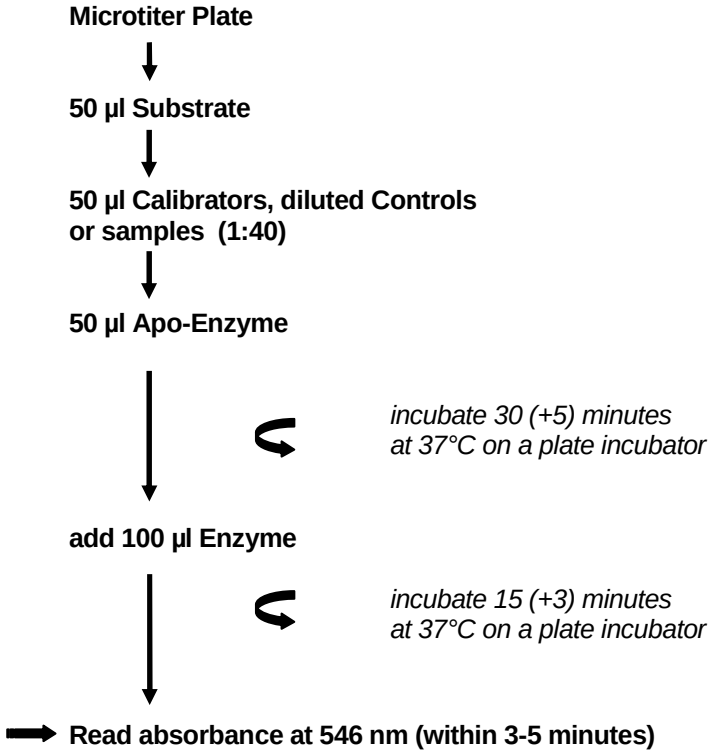
Vitamin B6 enzymatic



TIME TO RESULT: 50 MINUTES

APPENDIX IV
SYMBOLS

Symbol	Explanation
	Use By
REF	Catalogue number
LOT	Batch code
	Temperature limitation
	Consult Instructions for Use-
	Contains sufficient for <n> tests

Symbol	Explanation
[CONTROL|L]	Control Low
[CONTROL|N]	Normal Control
[CAL|A]	Calibrator A
[CAL|B]	Calibrator B
[CAL|0]	Calibrator 0
[SUB]	Substrate
[APOE]	Apo-Enzyme
[E]	Enzyme
[DB]	Dilution Buffer
[EB]	Enzyme Buffer