Vitamin B6 enzymatic

Procedure KK-VB6-U

Intended Use

The Vitamin B6 Enzymatic Assay (KK-VB6-U) is intended for the determination of Pyridoxal 5'-Phosphate (PLP, vitamin B6) in EDTA plasma. For Research Use Only. Not 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 TOOS in the presence of horseradish peroxidase to obtain a quinoneimine (purple dye) the absorbance of which is measured at 546 nm (520-595 nm).

Manual Procedure

Reagents have to be adjusted to 18-28°C .

Dilute EDTA plasma and Controls 1:40 in Dilution Buffer

Pipett 50 µl Substrate R1 into each well

Pipett 50 µl Calibrators 0, 20, 200 nmol/L
into the respective wells

Pipett 50 µl Control low and normal (diluted)
into the respective wells

Pipett 50 µl diluted sample into the subsequent wells.

Pipett 50 µl Apo-Enzyme R2 into each well.

shake and incubate for 30 + 5 min at 37°C in a plate incubator

Pipett 100 µl Enzyme R3 into each well

shake and incubate for 15 + 3 min at 37°C in a plate incubator

Read OD at 546 nm (alternatively at 520-595 nm)

Use endpoint mode with two calibrators (20 and 200 nmol/L). Calibrator 0 is used as Blank. Have a standard curve created by using linear curve-fitting.

Special Equipment

Manual procedure:
Microtiterplate reader with a filter at 546 nm, (520-595 nm) incubation chamber at 37°C and software suitable for endpoint measurements.

Microtiterplates, e.g. NUNC Maxisorb F8

Pre-Analytics

Samples required:	~500 µl EDTA plasma dilute 1:40 in dilution buffer **Lipemic plasma:** Samples should be taken from fasting individuals due to interferences with the photometric determination. **Hemolytic plasma:** Slightly hemolytic samples can be used.
Sample collection:	Draw blood into EDTA venipuncture tubes
Sample storage:	at 2-8°C up to 12 h protected from light. at -20°C for at least 3 months

Kit components

	KK-VB6
Tests	100
Dilution Buffer	1 x 60 mL
Enzyme Buffer	1 x 13 mL
Substrate R1	1 x lyophilized
Apo-Enzyme R2	1 x lyophilized
Enzyme R3	2 x lyophilized
Calibrator Set	1 x 3 lyophilized
Control Set normal / high	1 x 2 lyophilized

Reconstituted reagents are stable for 2 months at 2-8 °C except of Apo-Enzyme, Calibrator, and Controls (undiluted) which are stable for 2 months at ≤-20°C; store in aliquots, if reagent is needed for more than 3 runs. Controls have to be diluted 1:40 prior to usage.

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Vitamin B6 enzymatic Characteristics KK-VB6-U

Assay Performance Data

Data have been established with the manual procedure on microtiterplates.

Dilution linearity	9-250 nmol/L
Spiking recovery	81-105 %

3 samples were spiked with increasing amounts of PLP and analysed in 3 runs.

Sensitivity

LoB:	< 7 nmol/L
LoD:	< 7 nmol/L
LoQ:	< 10 nmol/L
Repeatability	<10 %
Total precision	<15 %

Diluted EDTA samples n=5 were tested over a period of 20 work days in 2 runs per day.

Precision Profile

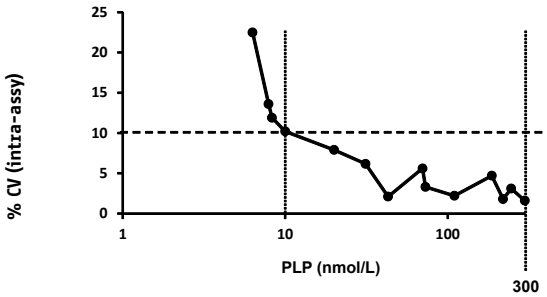

Figure 1: Precision Profile

Specificity of the Enzyme	Max. conc. tested nmol/L	Reactivity %
Component		
Pyridoxal (PL)	10'000	≤0.1 %
Pyridoxin (PN)	10'000	≤0.1 %
Pyridoxamine (PM)	10'000	≤0.1 %
4-pyridoxic acid (PA)	10'000	≤0.1 %
Pyridoxamine 5'-phosphate (PMP)	1200 10'000	≤0.2 % ≤0.8 %

Method Comparison HPLC vs Enzymatic

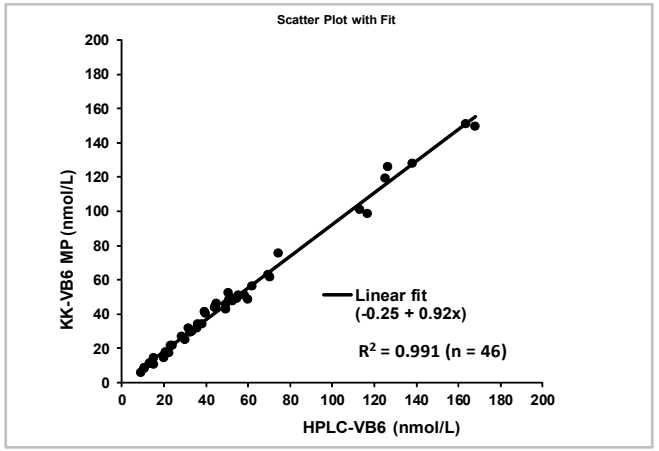

Figure 2: Method comparison with EDTA plasma samples

Interfering Substances

No interference is detected with the following substances up to the listed concentrations:

Lipemic samples: triglycerides: Intralipid®200 mg/dL; equivalent to 5.6 mmol/L triglycerides

Hemolytic samples: haemoglobin: 3.2 mmol/L; 500 mg/dL

Icteric samples: conjugated bilirubin: 360 µmol/L; 30 mg/dL, unconjugated bilirubin: 214 µmol/L; 12.5 mg/dL

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

For Research Use Only in the US and Canada

Vitamin B6 Enzymatic Assay is available for Research Use Only in the US and Canada for determination of Pyridoxal 5'-Phosphate in EDTA samples. Not for use in diagnostic procedures.

Ordering code:
KK-VB6-U 100 tests

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