Vitamin B6 enzymatic

**Intended Use**
The Vitamin B6 enzymatic assay (KK-VB6) is intended for the quantitative determination of Pyridoxal 5'-Phosphate (PLP, vitamin B6) in EDTA plasma. The BÜHLMANN Vitamin B6 enzymatic assay allows for detection of potential vitamin B6 deficiency or overdose.

**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 (H₂O₂) by the action of tyramine oxidase. The H₂O₂ reacts with 4-aminooantipyrine 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.

Pipett 100 µl Enzyme R3 into each well

shake and incubate for 30 + 5 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**

<table>
<thead>
<tr>
<th>Tests</th>
<th>KK-VB6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dilution Buffer</td>
<td>1 x 60 ml</td>
</tr>
<tr>
<td>Enzyme Buffer</td>
<td>1 x 13 ml</td>
</tr>
<tr>
<td>Substrate R1</td>
<td>1 x lyophilized</td>
</tr>
<tr>
<td>Apo-Enzyme R2</td>
<td>1 x lyophilized</td>
</tr>
<tr>
<td>Enzyme R3</td>
<td>2 x lyophilized</td>
</tr>
<tr>
<td>Calibrator Set</td>
<td>1 x 3 lyophilized</td>
</tr>
<tr>
<td>Control Set</td>
<td>1 x 2 lyophilized</td>
</tr>
</tbody>
</table>

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.
**Vitamin B6 enzymatic**

**Characteristics**

**KK-VB6**

### Assay Performance Data

Data have been established with the manual procedure on microtiter plates.

- **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

![Precision Profile](image)

**Figure 1: Precision Profile**

### Specificity of the Enzyme

<table>
<thead>
<tr>
<th>Component</th>
<th>Max. conc. tested (nmol/L)</th>
<th>Reactivity %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyridoxal (PL)</td>
<td>10'000</td>
<td>≤0.1 %</td>
</tr>
<tr>
<td>Pyridoxin (PN)</td>
<td>10'000</td>
<td>≤0.1 %</td>
</tr>
<tr>
<td>Pyridoxamine (PM)</td>
<td>10'000</td>
<td>≤0.1 %</td>
</tr>
<tr>
<td>4-pyridoxic acid (PA)</td>
<td>10'000</td>
<td>≤0.1 %</td>
</tr>
<tr>
<td>Pyridoxamine 5’-phosphate (PMP)</td>
<td>1200</td>
<td>≤0.2 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td>≤0.8 %</td>
</tr>
</tbody>
</table>

### Method comparison HPLC vs enzymatic

![Scatter Plot with Fit](image)

**Figure 2: Method comparison with EDTA plasma samples**

### Normal Values

<table>
<thead>
<tr>
<th>Apparently healthy adults</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median (nmol/L)</td>
<td>23</td>
</tr>
<tr>
<td>95th Percentile (nmol/L)</td>
<td>172.5</td>
</tr>
<tr>
<td>2.5th Percentile (nmol/L)</td>
<td>57.5</td>
</tr>
</tbody>
</table>

### Interfering Substances

- **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 in this study. Interferences cannot be excluded.

### Ordering code:

- **KK-VB6** 100 tests

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