HPLC Applications / LC-MS/MS Applications

Skeletal System

Oxidative Stress

Preventive Medicine

Cardiovascular System
Introduction

Immundiagnostik specialises in developing and producing innovative parameters and detection methods for use in clinical diagnostics and in medical research.

HPLC analysis is a well established method in laboratory diagnostics. The use of an internal standard and calibrator justifies single determinations in the daily routine work. Contrary to immunological assays, matrix effects can be recognized in HPLC and eliminated. The method is, from an economical view, suitable for the measurement of limited samples but it can also be automated for large sample series.

Immundiagnostik AG also offers all the components of the ready-to-use-applications as single reagents. This ensures optimal use of the assay components.

Please find more information about our monoclonal and polyclonal antibodies in our catalogue "IMMUNOCHEMICALS, ANTIBODIES, ANTIGENS" or on our website www.immundiagnostik.com
Adenosine

Adenosine is a ubiquitous, biologically important nucleoside which is a precursor of other biologically active molecules as well as a component of some co-factors. Moreover, it also has its own distinct physiological functions in the central nervous system, the cardiovascular system, the skeletal muscle and the immune system.

One of the principal intracellular actions of adenosine is inhibition of the enzyme phosphodiesterase. Extracellular adenosine has specific neuromodulatory actions on dopamine and glutamate.

Adenosine can act either as a hormone by binding to adenosine receptors or as an intracellular modulator after its translocation into the cell by membrane transport proteins. Four adenosine receptor subtypes have been identified. Notably, adenosine receptors are most widely expressed in the brain and the cardiovascular system, but they also are found in the most of the other tissues: respiratory tract, intestine, kidney, skeletal muscle, pituitary gland, uterus and gonads. Adenosine modulates several physiological effects by stimulating specific cell surface receptors. In addition, adenosine acts as an endogenous regulator of immune and inflammatory processes.

Adenosine exerts multifaceted effects on the heart and blood vessels and is involved in the regulation of the renal function. It works as a universal protective agent against hypoxia, ischemia, excitotoxicity, toxicities induced by other substances and trauma. It is also an effective and safe therapeutic medicine for paroxysmal tachycardias in adult and pediatric patients, with basic electrophysiologic properties of slowing conduction in atrioventricular nodes.

The measurement of urinary adenosine can contribute to evaluation of renal injury, metabolic disease or severe respiratory failure, as it was found that unfavorable pathophysiologic conditions are associated with appreciable elevation of adenosine.

**Indications**

- Evaluation of renal injury
- Metabolic diseases

<table>
<thead>
<tr>
<th>UV-Detection</th>
<th>254 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample volume</td>
<td>500 µl</td>
</tr>
<tr>
<td>Elution</td>
<td>Gradient</td>
</tr>
<tr>
<td>Matrix</td>
<td>Urine</td>
</tr>
<tr>
<td>Test principle</td>
<td>HPLC</td>
</tr>
<tr>
<td>Tests</td>
<td>96</td>
</tr>
<tr>
<td>Cat. No. Adenosine</td>
<td>KC8000</td>
</tr>
</tbody>
</table>

**Chromatogram Adenosine**
Glutathione (GSH/GSSG)

Glutathione is the most important cellular protection system against the toxic effects of metals such as Hg$^{2+}$ and MeHg. It is a tri-peptide of cysteine, glycine and glutamic acid. As an antioxidant, it can catch free radicals, reduce H$_2$O$_2$ and stabilize sulf-hydryl groups. H$_2$O$_2$ is transformed into oxygen and water with the aid of GSH-peroxidase, whereby GSH itself is oxidised to GSSG. The enzyme glutathione reductase reduces GSSG to active GSH.

Glutathione is transferred to xenobiotica via the enzyme glutathione-S-transferase. The water solubility of this substance is increased and the elimination facilitated. Intracellular redox regulation is impaired by these xenobiotics. The result is a competition between the excretion of GSSG and the GSH-xenobiotica-complex. This is the reason for the intracellular increase of the GSSG concentration. In addition, chlorpyrifos can block the reduction of GSSG, thus shifting the balance of GSH to GSSG. Due to this shift, cell functions of all organs are extremely affected and vitally threatened.

Patients suffering from chronic lymphatic oedema show a 20% lower GSH level in their erythrocytes compared to controls (1.68 mmol/l cells compared to 2.04 mmol/l). The GSSG concentration, however, is increased by about 5% (59.25 µmol/l cells compared to 56.79 µmol/l).

**Indications**

- Oxidation status (e.g. in diabetes)
- Studies of oxidative stress in the cell
- Stress through increased oxygen turnover under high-performance conditions

**Baseline Values Glutathione (GSH/GSSG)**

<table>
<thead>
<tr>
<th>Category</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDTA-whole blood</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>763 – 1191 µmol/l</td>
</tr>
<tr>
<td>Reduced</td>
<td>620 – 970 µmol/l</td>
</tr>
</tbody>
</table>

**Flow rate** 1 - 1.5 ml/min  
**Detection**  Fluorescence: Ex: 385 nm; Em: 515 nm  
**Sample volume** 100 µl  
**Elution** isocratic  
**Matrix** EDTA-whole blood  
**Tests** 100  
**Cat. No. Glutathione** KC1800
Homocysteine

Homocysteine is established as a risk factor for the development of arteriosclerotic and thrombovascular diseases.

In pediatrics this parameter also has relative importance in prenatal screening for the diagnosis of congenital homocysteinuria. A vitamin dependent hyperhomocysteinaemia can be distinguished from the genetically dependent form through the measurement of the vitamins B<sub>6</sub>, B<sub>12</sub> and folic acid.

Dialysis patients present an especially high-risk group because of associated arteriosclerosis. For this reason monitoring of the homocysteine and Vitamin B<sub>12</sub> levels belongs to the routine examinations for this group. Recent studies show that age related lethargy can often be attributed to an increased homocysteine level and a reduced Vitamin B<sub>12</sub> level, respectively. The therapy of this deficient state leads to the increased mobility and mental activity of these patients.

**Indications**

- Coronary artery diseases
- Homocysteinuria
- Hyperhomocysteinaemia
- Prenatal screening

<table>
<thead>
<tr>
<th>Baseline Values Homocysteine</th>
<th>Serum / Plasma &lt; 15 µmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow rate</td>
<td>0.75 ml/min</td>
</tr>
<tr>
<td>Detection</td>
<td>Fluorescence: Ex.: 385 nm; Em.: 515 nm</td>
</tr>
<tr>
<td>Sample volume</td>
<td>50 µl</td>
</tr>
<tr>
<td>Matrix</td>
<td>Serum, Plasma</td>
</tr>
<tr>
<td>Elution</td>
<td>isocratic</td>
</tr>
<tr>
<td>Tests</td>
<td>100</td>
</tr>
<tr>
<td>Cat. No. Homocystine</td>
<td>KC2801</td>
</tr>
</tbody>
</table>

**Running time <= 5 min - doubling the sample turnover rate**

**Sample volume: 50 µl**

**Sample preparation time: 10 min**

**Higher column stability through pH-value optimization**

Chromatogram Homocysteine

References

Hydroxy-Pyridinium-Crosslinks

The covalent cross-linking of collagen is necessary for the stability of the collagen network in the connective tissue. In spite of the fact that all the connective tissue which contains collagen has these crosslinks, there are still very tissue-specific characteristics. If the content of hydroxylated lysine in the telo peptide region of the collagen molecule is high, then tri-functional 3-hydroxy-pyridinium crosslinks are formed and these consist of pyridinolin (PYD) and deoxypyridinolin (DPD). This is the case in bones and in cartilage. However deoxypyridinolin is found almost exclusively in bones, hardly ever in cartilage or in intervertecal discs. Detectable amounts of deoxypyridinolin can be found in the aorta or in ligaments. Because bones have a considerably higher metabolism, the secretion of crosslinks is very specific to bones.

The cross-linking of collagen represents a maturing process outside of the cells and therefore the release of crosslinks offers a very specific marker for the deterioration of mature collagen from bones. Therefore crosslinks should be defined as a specific marker for bone resorption.

During the aging process, one finds an elevated secretion of crosslinks. Mild kidney failure has no influence on the crosslinks.

On the other hand, there are noticeable fluctuations dependant on the circadian rhythm and the highest secretion of crosslinks is found during the night. In order to compensate for varying diuresis, crosslinks are related to creatinine concentrations.

Crosslinks concentration is lowered by exercise. Post-menopausal women have an elevated level of crosslinks, whereas women with post-menopausal osteoporosis have an even higher crosslinks secretion. When estrogen therapy is given, crosslinks in women with osteoporosis decrease (Seibel et al., 1994).

Post-menopausal women with high Crosslinks show a high decrease in bone density over time. This secretion of Crosslinks provides, apart from the bone mass, a good predictor for future bone fractures (Garnero et al., 1995).

Urinary crosslinks can act as an indicator for the choice of a suitable therapy for osteoporosis. Therapy with calcitonin was only effective in women at the stage of increased bone remodeling (Civitelli et al., 1988).

The measurement of crosslinks in urine can also be used for the monitoring of therapy (Garnero et al., 1994). Patients demonstrating a strong decrease in crosslinks during bisphosphonate therapy consequently had better progress where their bone mass was concerned.

As can be expected, the secretion of crosslinks is also elevated in patients with other diseases which lead to resorption of bones: Paget’s disease, primary hyperparathyroidism, hyperthyreosis and bone metastases.

**Indications**

- Diagnosis of bone metabolism disturbances which are accompanied by high bone deterioration (osteoporosis, primary hyperparathyroidism, hypogonadism, hyperthyreosis, bone metastases)
- Evaluation of the risk of bone fractures in osteoporosis
- Monitoring of therapy
Baseline Values Hydroxy-Pyridinium Crosslinks

Urine:
38.4 ± 18.4 nmol PYD / mmol Creatinin
10.3 ± 5.3 nmol DPD / mmol Creatinin

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow rate</td>
<td>1 – 1.5 ml/min</td>
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<tr>
<td>Detection</td>
<td>Fluorescence:</td>
</tr>
<tr>
<td></td>
<td>Ex.: 290 nm; Em.: 400 nm</td>
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<tr>
<td>Sample volume</td>
<td>1 ml</td>
</tr>
<tr>
<td>Elution</td>
<td>isocratic</td>
</tr>
<tr>
<td>Matrix</td>
<td>Urine</td>
</tr>
<tr>
<td>Tests</td>
<td>100</td>
</tr>
<tr>
<td>Cat. No. PYD / DPD</td>
<td>KC3201</td>
</tr>
</tbody>
</table>

Chromatogram Hydroxy-Pyridinium Crosslinks (PYD / DPD)

References
Zittermeann et al. Calcif Tissue Int 2002;70:16-21
Malondialdehyde (MDA)

Marker for oxidative damage
5 minutes run time

As a result of the oxidation of unsaturated fatty acids of the cell membranes, a wide spectrum of various hydroperoxides can be produced in the organism. These fatty acid derivatives are chemically unstable and are quickly transformed into aldehydes. Amongst these is malondialdehyde, which has the property of being able to cross-link proteins and lipids.

The MDA levels in the serum of patients with terminal kidney failure (dialysis) (4.05 ± 1.7 µmol/l), with septic shock (3.4 ± 2.3 µmol/l), with a high-risk pregnancy (9.4 ± 4.6 µmol/l) as well as female volunteers taking contraceptives (8.37 ± 3.57 µmol/l) are significantly increased compared to baseline values (1.97 ± 0.41 µmol/l).

A comparison of patients with "unstable angina (UA)" and patients with myocardial infarction (MI) shows that the MDA level in the plasma of patients with UA increases until the 5th day and then decreases until the 12th day. An immediate increase of the concentration can be seen in MI patients, followed by a decrease within the following 12 days.

Indications

- Indicator for lipid peroxidation. Useful for biological, medical and nutritional studies
- Biochemical marker for oxidative stress

References

Winnefeld et al. (1995) GIT Labor Medizin 18: 355-357
**Porphyins**

The *Porphyins* are precursors of proteides which include hemoglobin, myoglobin and the cytochromes. They play an important role in oxygen metabolism.

There are several genetic defects of heme biosynthesis in liver and erythroid cells. In all of them an increase of Porphyins is seen. These diseases are therefore known as porphyria.

**Indications**

- Hereditary hepatic porphyria (porphyria variegata)
- Porphyria cutanea tarda in chronic liver disease and alcoholic liver syndrome
- Hepatic porphyria in prostate and liver tumors
- Acute and chronic lead poisoning

**Measurement of total porphyrins in a single run**

**Running time:** 25 minutes

**Very simple sample preparation**

**Highly stable controls**

**Separation of coproporphyrin I and III**

<table>
<thead>
<tr>
<th>Flow rate</th>
<th>0.5 – 0.8 ml/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detection</td>
<td>Fluorescence: Ex.: 400 nm; Em.: 620 nm</td>
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<tr>
<td>Sample volume</td>
<td>1ml</td>
</tr>
<tr>
<td>Elution</td>
<td>Gradient</td>
</tr>
<tr>
<td>Matrix</td>
<td>Urine</td>
</tr>
<tr>
<td>Tests</td>
<td>100</td>
</tr>
<tr>
<td>Cat. No. Porphyrins</td>
<td>KC2601</td>
</tr>
</tbody>
</table>

Chromatogram Porphyrins:

1. 8-carboxyl Porphyrin (Uro-)
2. 7-carboxyl Porphyrin (Hepta-)
3. 6-carboxyl Porphyrin (Hexa-)
4. 5-carboxyl Porphyrin (Penta-)
5. 4-carboxyl Porphyrin (Copro-) I
6. 4-carboxyl Porphyrin (Copro-) III
7. Mesoporphyrin IX
Ubiquinone / Coenzyme Q\textsubscript{10}

Ubiquinone was first isolated in the 50’s by Prof. Green’s group (Wisconsin). The function was investigated by Prof. Mitchel, who received the Nobel price for his research on the oxidative phosphorylation pathway.

Ubiquinone is a coenzyme which is represented in every cell and the whole metabolism. It is made up of a chinonic ring and an isoprenic sidechain. In humans Ubiquinone can be synthesized and absorbed through nutrition. Ubiquinone has two different physiological functions:

- Component of the energy metabolism
- Radical scavanger

3 Mol ATP is generated during the reduction of oxygen in the oxidative phosphorylation. In this reduction, electrons are transferred from NADPH to oxygen via 6 different redox systems. Ubiquinone is the least abundant redox system in the membrane of the mitochondria. Because of the low amount, it is the speed controlling redox-system in the energy metabolism. Normally the amount of ubiquinone is sufficient, but with growing age and exposure to sunlight it is reduced to 50 %. Patients under treatment with cholesterol reducers show a dose related decrease of Coenzyme Q10.

Ubiquinone has a high amount of carbon doublebonds and therefore a higher potential of reduction than Vitamin C or Vitamin E. Therefore it is the first line of defense against free radicals. Therefore ubiquinone is an optimal stabilizer of the ion channels of the membranes.

**Indications**

- Determination of Coenzyme Q10/ Ubiquinone status (especially under treatment with cholesterol reducers as stains)
- Cardiovascular disease
- Carcinogenesis
- Aging

*First commercially obtainable kit for the determination of Ubiquinone*

*Internal standard runs in the chromatogram after Q\textsubscript{10}; therefore no false-negative results*
Baseline Values Ubiquinone / Coenzyme Q10

EDTA whole blood / EDTA plasma: 0.67 – 0.99 μg/ml

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow rate</td>
<td>0.8 - 1.2 ml/min</td>
</tr>
<tr>
<td>Detection</td>
<td>UV: 275 nm</td>
</tr>
<tr>
<td>Sample volume</td>
<td>100 µl</td>
</tr>
<tr>
<td>Elution</td>
<td>isocratic</td>
</tr>
<tr>
<td>Matrix</td>
<td>EDTA whole blood, EDTA plasma, serum</td>
</tr>
<tr>
<td>Tests</td>
<td>100</td>
</tr>
<tr>
<td>Cat. No. Ubiquinone/Coenzyme Q₁₀</td>
<td>KC1700</td>
</tr>
</tbody>
</table>

Chromatogram Ubiquinone / Coenzyme Q₁₀

References
Vitamin A/E
(Retinol/Tocopherol)

Vitamin A (Retinol) is essential for the visual processes and keeps the skin and mucosa healthy. A lack of Vitamin A will reduce visual power, especially the quick adaptation from light to darkness. Serious Vitamin A deficiency can lead to total blindness.

An excess of Vitamin A causes headaches, skin changes, liver damage, painful skeletal alterations and possible fetal damage.

Vitamin E (Tocopherol) is a natural antioxidant and protects various vitamins and unsaturated fatty acids against oxidation. Vitamin E can be stored in large amounts in adipose tissue. A lack of Vitamin E can be caused by a malfunction in digestion or resorption of fatty acids.

**Indications**

- Disturbed lipid resorption
- Cystic pancreatitis
- Disturbance of bone growth
- Visual disturbance
- Alteration of retina
- Degeneration of testes and ovaries
- Reduction of heart attack risk

**Baseline Values Vitamins A / E**

| Vitamin A (Serum) | 200 - 800 µg/l |
| Vitamin E (Serum) | 3 - 14 mg/l |

**Chromatogram Vitamin A/E**

<table>
<thead>
<tr>
<th>Chromatogram Vitamin A/E</th>
</tr>
</thead>
</table>

**Very simple sample preparation**
(precipitation step for the separation of higher molecular substances)

**Internal standard**

**Total running time <15 minutes**

Vitamin A/E (HPLC)

<table>
<thead>
<tr>
<th>Test</th>
<th>0.8 ml/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow rate</td>
<td>0.8 ml/min</td>
</tr>
<tr>
<td>UV-Detection</td>
<td>Vitamin A: 325 nm; Vitamin E: 300 nm</td>
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<tr>
<td>Sample volume</td>
<td>250 µl</td>
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<tr>
<td>Matrix</td>
<td>Serum, Plasma</td>
</tr>
<tr>
<td>Elution</td>
<td>isocratic</td>
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<tr>
<td>Tests</td>
<td>100</td>
</tr>
<tr>
<td>Cat. No.</td>
<td>KC1600</td>
</tr>
</tbody>
</table>
Vitamin B\textsubscript{1}
(Thiamine pyrophosphate)

Thiamine pyrophosphate is the bioactive component of Vitamin B\textsubscript{1}. It acts as a co-factor on enzymes and plays an important role in carbohydrate and amino acid metabolism. An important reaction is the oxidative carboxylation. Thiamine stimulates nerve cells. In addition, it stimulates the fatty acid and cholesterol synthesis in nerve tissues.

Serious Vitamin B\textsubscript{1} deficiency, in conjunction with a low protein diet, leads to the illness known as Beri Beri. Even more serious in civilized society is the lack of Vitamin B\textsubscript{1} following artificial nutrition, which can lead to considerable damage in brain functions.

Other diseases caused by lack of thiamin are Wernicke-encephalopathy, Korsakow-syndrome and several forms of Landry’s paralysis.

**Indications**

- Measurement of the metabolic active Vitamin B\textsubscript{1}
- Vitamin B\textsubscript{1} supply in artificial nutrition
- Disorders of the amino acid metabolism
- Malabsorption caused by alcohol abuse
- Suspicion of neuritis

**Baseline Values Vitamin B\textsubscript{1}
EDTA-whole blood 32 - 95 ng/ml**

<table>
<thead>
<tr>
<th>Vitamin B\textsubscript{1} (HPLC)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow rate</td>
<td>0.8 - 1.2 ml/min</td>
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<tr>
<td>Detection</td>
<td>Fluorescence:</td>
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<td>Ex.: 365 nm; Em.: 440 nm</td>
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<tr>
<td>Sample volume</td>
<td>50 µl</td>
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<tr>
<td>Elution</td>
<td>isocratic</td>
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<tr>
<td>Matrix</td>
<td>EDTA-whole blood</td>
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<tr>
<td>Tests</td>
<td>100</td>
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<tr>
<td>Cat. No.</td>
<td>KC2201</td>
</tr>
</tbody>
</table>

**No post-column derivatisation**

**Eluent can be recirculated**

**Very simple sample preparation**

**Incubation time 10 minutes + 5 minutes**

**Chromatogram Vitamin B\textsubscript{1}**
Vitamin B₆
(Pyridoxal-5-phosphate)

Vitamin B₆ is the collective term for pyridoxin, pyridoxal, pyridoxamin and their phosphates. All forms can be metabolised to the active form pyridoxal-5-phosphate. It functions as a coenzyme in protein metabolism and is indispensable in more than 50 reactions. Vitamin B₆ participates in blood formation as a component of the hem forming enzyme. Furthermore, Vitamin B₆ is a contributing factor in the formation of neurotransmitters and biogenic amines (e.g. histamine).

Symptoms of a Vitamin B₆ deficiency are, amongst others, disturbances in protein biosynthesis, muscle dystrophy, skin lesions (scaling, hyperpigmentation) and neurological disorders (irritability, depression, paralysis). Recently, Vitamin B₆ has been designated as an important risk factor for myocardial infactions, peripheral vascular diseases and arteriosclerosis, especially in connection with the regulation of the homocysteine metabolism.

**Indications**

- Determination of the Vitamin B₆ state (especially in dialysis patients)
- Homocysteinaemia (vitamin dependent)
- Skin lesions
- Mobility disorders
- Anaemia / depression (especially senile depression)

**Baseline Values Vitamin B₆**

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Value Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma / serum</td>
<td>4.3 - 17.5 ng/ml</td>
</tr>
</tbody>
</table>

**Vitamin B₆ (HPLC)**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow rate</td>
<td>1 - 1.5 ml/min</td>
</tr>
<tr>
<td>UV-Detection</td>
<td>Fluorescence:</td>
</tr>
<tr>
<td></td>
<td>Ex.: 320 nm; Em.: 415 nm</td>
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<tr>
<td>Sample volume</td>
<td>200 µl</td>
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<tr>
<td>Elution</td>
<td>Isocratic</td>
</tr>
<tr>
<td>Matrix</td>
<td>Serum, Plasma</td>
</tr>
<tr>
<td>Column</td>
<td>Bischoff Prontosil Eurobond (125 x 4 mm)</td>
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<tr>
<td>Tests</td>
<td>100</td>
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<td>Cat. No.</td>
<td>KC2100</td>
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**Chromatogram Vitamin B₆**

**Vitamin B₆ (HPLC)**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>UV-Detection</td>
<td>Fluorescence:</td>
</tr>
<tr>
<td></td>
<td>Ex.: 320 nm; Em.: 415 nm</td>
</tr>
<tr>
<td>Sample volume</td>
<td>200 µl</td>
</tr>
<tr>
<td>Elution</td>
<td>Isocratic</td>
</tr>
<tr>
<td>Matrix</td>
<td>EDTA-whole blood, Serum, Plasma</td>
</tr>
<tr>
<td>Tests</td>
<td>100</td>
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<tr>
<td>Cat. No.</td>
<td>KC2150</td>
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</tbody>
</table>

References
**Vitamin C**
*(Ascorbic acid)*

Ascorbic acid, being a part of the antioxidative defense system, is found in both the cytosol and extracellular space. Depending on the concentration and the availability of transitional metals, it has both antioxidative and prooxidative features. The antioxidative effect dominates, especially in extracellular space. Since it acts through formation of semi-dehydro-ascorbate and dehydro-ascorbate respectively, as an electron donor transferring hydrogen to acceptor substances by reversibility, ascorbic acid has strong reducing effects.

Vitamin C makes a contribution to the antioxidative defense system in two different ways. On the one hand, it reacts with the reactive oxygen species, especially peroxide radicals. On the other hand, ascorbic acid regenerates α-tocopherol (Vitamin E). Vitamin C also has a pro-oxidative effect in combination with transition metals. It catalyses the reduction of Fe\(^{3+}\) to Fe\(^{2+}\). The created bivalent iron ions react faster with \(\text{H}_2\text{O}_2\). Therefore, the formation of OH\(^-\) radicals is supported through the Haber-Weiss-Reaction.

\[
\begin{align*}
\text{Fe-Reduction:} & \quad \text{O}_2 + \text{Fe}^{3+} & \rightarrow & \text{O}_2 + \text{Fe}^{2+} \\
\text{Fenton-Reduction:} & \quad \text{H}_2\text{O}_2 + \text{Fe}^{2+} & \rightarrow & \text{OH}^- + \text{OH}^- + \text{Fe}^{3+} \\
\text{Haber-Weiss-Reduction:} & \quad \text{O}_2^- + \text{H}_2\text{O}_2 & \rightarrow & \text{O}_2 + \text{OH}^- + \text{OH}^-
\end{align*}
\]

Due to the very small concentration of free transition metals in biological tissues, the antioxidative features are predominant. As a result of increased oxidative stress, the level of Vitamin C is reduced in various syndromes, e.g. the level of Vitamin C in blood from HIV positive patients is significantly lower. The content in blood plasma falls from 75.7 µmol/l to 40.7 µmol/l.

Smoking causes a high consumption of Vitamin C in the blood plasma. Protein thiols are oxidised and after the Vitamin C pool has been depleted, lipid peroxidation begins.

<table>
<thead>
<tr>
<th>Baseline Values Vitamin C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lithium heparinate plasma: 4 – 20 mg/l</td>
</tr>
</tbody>
</table>

**Simple sample preparation**

Prepared sample material is conservable: at room temperature and 2-8 °C up to 24 hours; at –20 °C up to 8 weeks

**Stable controls**

Mobile phase can be circulated

**Indications**

- Determination of Vitamin C status (protection from oxidation)
- Vitamin C deficiency in heavy smokers

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**References**

25(OH) Vitamin D₃

Vitamin D₃ and Vitamin D₂ are the most important D vitamins. Contrary to Provitamin D₂ which must be supplied through nutrition, Provitamin D₃ can also be produced in the liver. Vitamin D₃, whether it is produced in the skin or supplied through nutrition together with Vitamin D₂, is bound to Vitamin D binding protein in plasma and transported to the liver. There it is hydroxylated in position 25 to form 25-OH D. More than 95% of the circulating 25-OH Vitamin D is 25-OH D₃. 25-OH D₂ can only be measured in patients under medication with Vitamin D₂.

The HPLC application is normally used to determine 25-OH D₃. By using another calibrator it is possible to determine 25-OH D₂.

Indications

- Diagnosis of hyper- and hypovitaminosis
- Control of the Vitamin D storage supply (especially in dialysis patients)
- Osteoporosis screening

Baseline Values 25 (OH) Vitamin D₃

<table>
<thead>
<tr>
<th>Serum:</th>
<th>15 – 150 nmol/l</th>
</tr>
</thead>
</table>

Flow rate | 1 – 1.5 ml/min |
UV-Detector | 264 nm |
Sample volume | 500 µl |
Elution | isocratic |
Matrix | Serum, Plasma |
Tests | 100 |
Cat. No. 25-OH Vitamin D₃ | KC3400 |

New:

- Very stable and reproducible analytical procedure
- Reference method for 25-OH Vitamin D determination
- Specific for 25-OH D₃ and/or 25-OH D₂

Chromatogram Vitamin D₃
Vitamin K

Vitamin K, which was first discovered in 1929, takes its name from the word "Koagulation". Vitamin K is a co-factor for gamma-carboxylase, an enzyme which is essential for the synthesis of coagulation factors (II, VII, IX, X) and anti-coagulants (proteins C, S und Z).

Since the discovery of osteocalcin it has become evident that Vitamin K plays an important role in bone metabolism. At present three bone matrix proteins which contain gamma-carboxy glutamic acid are known: osteocalcin (also called bone protein), matrix Gla protein, and protein S (Szulc and Delmas 1995).

Vitamin K₁ (phyllochinon) and Vitamin K₂ (menachinone) differ from each other in their chemical structure (side chain) both have, however, identical biological activity. Vitamin K₁ originates from green plants (cabbage, cauliflower, spinach) and the intestine. Vitamin K₂ is produced in bacteria in the intestinal flora, however its relevance in Vitamin K supply is assumed to be minimal. Risk factors for Vitamin K deficiencies are: malabsorption (bile tract illnesses, pancreas failure, chronic inflammatory bowel diseases, bowel resection), dietary deficiencies, breast feeding without supplements and antibiotic therapy.

Lowered Vitamin K levels are also found in aging women (Hodges et al. 1990). Vitamin K is not only stored in the liver but also in the bones. Vitamin K can accelerate bone fracture healing (Bouckaert and Said 1960), whereas Vitamin K antagonists delay the healing of bone fractures (Dodds et al. 1984).

Numerous, experimental data prove that Vitamin K affects osteoblast function, that it can delay osteoporosis induced by ovariectomy and that in Vitamin K deficiencies skeletal development is retarded (Szulc and Delmas 1995).

Patients with thigh fractures near the hips show decreased Vitamin K levels (Hart et al. 1985, Hodges et al. 1991, Hodges et al. 1993). Vitamin K improves the gamma-carboxylation of osteocalcin, which is an indicator for the formation of new bone.

Vitamin K seems to have a protective effect on the bone mass of patients with osteoporosis and renal osteopathy. In many studies, the treatment with Vitamin K antagonists resulted in the loss of bone mass.

A part of the effect of Vitamin K deficiency is due to the formation of incompletely carboxylated osteocalcin, which is found in an increased amount in patients who have been treated with Vitamin K antagonists. In a prospective study of older women with an elevated level of incompletely carboxylated osteocalcin, a considerably increased risk of thigh fractures in the region of the hips was determined (Szulc et al. 1994). Supplementation with Vitamin D in post-menopausal women with osteoporosis resulted in an improved carboxylation of osteocalcin (Douglas et al. 1995).

Indications

- Deficiencies of Vitamin K dependent coagulation factors
- Infants with an increased risk of Vitamin K deficiency
- Examination of bone metabolism disorders
- Senile osteoporosis

Calibration through internal standards
Postcolumn reduction reactor is included in the first kit
Reduction reactor can be refilled
Baseline Values Vitamin K₁

Serum/Plasma: 0.09 – 2.12 ng/ml

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
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</thead>
<tbody>
<tr>
<td>Flow rate</td>
<td>1-1.2 ml/min</td>
</tr>
<tr>
<td>Detection</td>
<td>Fluorescence: Ex.: 248nm Em.: 418nm</td>
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<tr>
<td>Sample volume</td>
<td>1 ml</td>
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<tr>
<td>Elution</td>
<td>isocratic</td>
</tr>
<tr>
<td>Matrix</td>
<td>Serum, Plasma</td>
</tr>
<tr>
<td>Tests</td>
<td>100</td>
</tr>
<tr>
<td>Cat. No. Vitamin K₁</td>
<td>KC2400</td>
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</tbody>
</table>

Chromatogram Vitamin K₁

References
Hodges et al. (1991) Bone 12: 387-389
Hodges et al. (1993) J Bone Min Res B: 1241-1245
Bouckaert et al. (1960) Nature 185: 849
Dodds et al. (1984) Calcif Tissue Int 36: 233-238
Hart et al. (1985) J Clin Endocrinol Metab 60: 1268-1269
Szulc et al. (1994) J Clin Invest 91: 1769-1774
Douglas et al. (1995) Bone 17: 15-20
Neurosteroids are formed from cholesterol via pregnenolone and progesterone. Pregnenolone and progesterone are precursors not only of the synthesis of gluco- and mineral corticoids in the adrenal gland and of the sexual hormones in the gonads or placenta, but also of neurosteroids in the central nervous system, i.e., pregnenolone is an endogenous, naturally produced steroid that is a precursor of many body hormones.

Numerous studies have demonstrated that neurosteroids interact with neurotransmitter receptors, influence the brain excitability, thereby functioning as potent allosteric modulators of several neurotransmitter receptors: the endogenous neurosteroid allopregnanolone, a positive modulator of the γ-aminobutyric acid type A (GABAA) receptor complex, activates the GABAA receptor functions lowered by various stresses, and has anxiolytic and anti-stress effects. In contrast, negative modulators, like dehydroepiandrosterone sulfate and pregnenolone sulfate, inhibit almost completely the GABAA-receptor.

Due to their lipophility, most of the free steroids can easily pass the blood-brain-barrier. A number of steroids are conjugated as sulfate or fatty acid esters and occur in concentrations much higher than these of the free steroids. In contrast to the free steroids, they can not pass through the blood-brain barrier and represent the active forms involved in the control of various physiological and pathophysiological processes.

Neurosteroids affect a number of processes in the central nervous system, which have a powerful effect on our thinking and feeling. Furthermore, neurosteroids influence the social and sexual behavior. At the same time, they are promising pharmaceutical targets for important indications like epilepsy, anxiety disorders and dementia. For these reasons, the determination of neurosteroids, especially of pregnenolone and pregnenilone sulfate, is expected to be helpful for understanding the roles of neurosteroids, for diagnosis of psychic or mental disorders and for the development of new steroidal therapeutic agents.

**Indications**

- Cognitive ability
- Weakness of memory
- Mood swings like depression

<table>
<thead>
<tr>
<th>Method</th>
<th>LC-MS/MS</th>
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<tbody>
<tr>
<td>Sample volume</td>
<td>200 µl</td>
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<tr>
<td>Matrix</td>
<td>EDTA-Plasma, Serum</td>
</tr>
<tr>
<td>Tests</td>
<td>100 Determinations</td>
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<tr>
<td>Modus</td>
<td>MRM</td>
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<td>Polarity</td>
<td>ESI negative</td>
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<tr>
<td>Cat. No.</td>
<td>KM2000</td>
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</tbody>
</table>

References
Mehta AK et al. (1999) Brain Res Brain Res Rev 29:196-217
Park-Chung et al. (1999) Brain Res 830:72-87
Hu et al. (1987) Proc Natl Acad Sci USA 84: 8215-8219
Please send me the following informative material:

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- Manuals of these products:
- 
- Brochures:
  - Skeletal System / Bone metabolism
  - Cardiovascular and Renal Systems
  - Gastrointestinal Diseases
  - Orthomolecular Medicine
  - Molecular Biology
  - Oxidative Stress

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