Glutamat Kit

For the determination of glutamate in human EDTA plasma and serum

Nur zu Forschungszwecken / For research use only

Gültig ab / Valid from 12.03.2013

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Glutamate Kit

*For the determination of glutamate in human EDTA plasma and serum*

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REF K 7731
1. INTENDED USE

This enzyme test is intended for the determination of glutamate in human EDTA plasma and serum. It is for research use only.

2. INTRODUCTION

Glutamic acid is a non-essential amino acid. The salts of glutamic acid are known as glutamates. Glutamate plays a central role in cellular metabolism. A significant process in amino acid degradation is transamination, in which the amino group of an amino acid is transferred to an α-ketoacid, typically catalysed by a transaminase. A very common α-keto acid is α-ketoglutarate, an intermediate in the citric acid cycle. Vice versa, transamination of α-ketoglutarate gives glutamate. The resulting α-ketoacid products pyruvate and oxaloacetate are key components in fundamental processes such as glycolysis, gluconeogenesis and also the citric acid cycle.

Glutamate also plays an important role in the body's disposal of excess or waste nitrogen. Glutamate undergoes deamination, an oxidative reaction catalysed by glutamate dehydrogenase.

In the vertebrate nervous system Glutamate is the most abundant excitatory neurotransmitter. At chemical synapses it binds to glutamate receptors, such as the NMDA receptor. Because of its role in synaptic plasticity, glutamate is involved in cognitive functions like learning and memory in the brain.

Glutamate is rapidly removed from the extracellular space by Glutamate transporters in neuronal and glial membranes. In brain injury or disease, they can work in reverse and excess glutamate can accumulate outside cells which leads to neuronal damage and eventual cell death. This excitotoxicity due to glutamate occurs as part of the ischemic cascade and is associated with stroke and diseases like amyotrophic lateral sclerosis, lathyrism, autism, some forms of mental retardation and Alzheimer's disease.

Moreover, in the brain Glutamate also serves as the precursor for the synthesis of the inhibitory neurotransmitter GABA in GABA-ergic neurons.

Free glutamic acid is present in a wide variety of foods and is often used as a food additive and flavour enhancer in the form of its sodium salt, monosodium glutamate.
3. **PRINCIPLE OF THE TEST**

This assay is a photometric test intended for the determination of L-glutamate by enzymatic dehydration, in which NAD$^+$ is transformed to NADH.

In this reaction L-glutamate is oxidized to α-ketoglutarate by reducing NAD$^+$ to NADH. This reaction can be measured at 340 nm and it is proportional to the amount of oxidized L-glutamate.

4. **MATERIAL SUPPLIED**

<table>
<thead>
<tr>
<th>Catalog No</th>
<th>Content</th>
<th>Kit Components</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>K7731MTP</td>
<td>PLATE</td>
<td>Microtiter plate</td>
<td>12 x 8 wells</td>
</tr>
<tr>
<td>K7731ST</td>
<td>STD</td>
<td>Standards, ready for use</td>
<td>4 x 1 vial</td>
</tr>
<tr>
<td>K7731KO</td>
<td>CTRL 1</td>
<td>Controls, ready for use</td>
<td>2 x 1 vial</td>
</tr>
<tr>
<td>K7731KO</td>
<td>CTRL 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K7731AP</td>
<td>ASYBUF</td>
<td>Assay buffer, ready for use</td>
<td>16 ml</td>
</tr>
<tr>
<td>K7731RP</td>
<td>REABUF</td>
<td>Reaction buffer, lyophilized</td>
<td>2 x 1 vial</td>
</tr>
<tr>
<td>K7731EB</td>
<td>ENZ B</td>
<td>Glutamate dehydrogenase, concentrate</td>
<td>2 x 1 vial</td>
</tr>
</tbody>
</table>

5. **MATERIAL REQUIRED BUT NOT SUPPLIED**

- Ultra pure water*
- Precision pipettors and disposable tips to deliver 10-1000 μl
- Foil to cover the microtiter plate
- A multi-channel dispenser or repeating dispenser
- Centrifuge capable of 3000 x g
- Vortex-Mixer
- Standard laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader at 340 nm
- Heated incubator at 37°C

* Immundiagnostik AG recommends the use of Ultra Pure Water (Water Type 1; ISO 3696), which is free of undissolved and colloidal ions and organic molecules (free of particles > 0.2 μm) with an electrical conductivity of 0.055 μS/cm at 25°C (≤18.2 MΩ cm).
6. PREPARATION AND STORAGE OF REAGENTS

- To run assay more than once, ensure that reagents are stored at conditions stated on the label. Prepare only the appropriate amount necessary for each assay. The kit can be used up to 2 times within the expiry date stated on the label.

- Store standards (STD) and controls (CTRL) frozen at -20°C, thaw before use in the test and mix well. Re-freeze standards and controls immediately after use. They can be re-frozen up to 2 times.

- Dissolve the content of one vial of reaction buffer (REABUF) in 3 ml ultra pure water, mix well. Discard any remaining quantity after use. By providing two REABUF vials the kit can be separated into two performances.

- To the content of one vial of glutamate dehydrogenase (ENZ B) 2.6 ml assay buffer (ASYBUF) must be added, mix well. Discard any remaining quantity after use. By providing two ENZ B vials the kit can be separated into two performances.

- All other test reagents are stable until date of expiry (see label) when stored at 2-8°C.

7. PRECAUTIONS

- For research use only.

- Reagents should not be used beyond the expiry date shown on kit label.

8. SPECIMEN COLLECTION AND PREPARATION

EDTA plasma and serum

- Venous fasting blood is suited for this test system. As glutamate is temperature sensitive use fresh blood samples or store samples at -20°C immediately after collection. Handle blood samples cool until use in the test.

- Lipemic or hemolytic samples may give erroneous results and should not be used for analysis.

- If less than 50 μl of sample is provided we recommend diluting the sample 1:2 in SAMPLEBUF (25 μl sample + 25 μl SAMPLEBUF). This dilution factor must be considered in data evaluation.

- Samples with visible amounts of precipitates should be centrifuged.
9. **ASSAY PROCEDURE**

**Procedural notes**
- The assay should always be performed according to the enclosed manual.
- Do not interchange different lot numbers of any kit component within the same assay.
- Quality control guidelines should be observed.
- Incubation time, incubation temperature, and pipetting volumes of the different components are defined by the producer. Any variations of the test procedure that are not coordinated with the producer may influence the test results. Immundiagnostik AG can therefore not be held reliable for any damage resulting from this.

**Test procedure**

<table>
<thead>
<tr>
<th>Step</th>
<th>Instruction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Mark the positions of standards (STD)/controls (CTRL)/ samples (SAMPLE) in duplicate on a protocol sheet</td>
</tr>
<tr>
<td>2.</td>
<td>Take as many microtiter strips (PLATE) as needed from kit. Store unused strips covered at room temperature.</td>
</tr>
<tr>
<td>3.</td>
<td>Add <strong>2 x 50 μl of standards (STD) / controls (CTRL) / samples (SAMPLE)</strong> into the respective wells of the microtiter plate (PLATE)</td>
</tr>
<tr>
<td>4.</td>
<td>Add <strong>50 μl of ultra pure water</strong> into each well.</td>
</tr>
<tr>
<td>5.</td>
<td>Add <strong>100 μl of assay buffer (ASYBUF)</strong> into each well</td>
</tr>
<tr>
<td>6.</td>
<td>Add <strong>50 μl of reaction buffer (REABUF)</strong> into each well, and determine absorption immediately with an ELISA reader at <strong>340 nm (OD&lt;sub&gt;BLANK&lt;/sub&gt;)</strong>.</td>
</tr>
<tr>
<td>7.</td>
<td>Add <strong>50 μl of diluted glutamate dehydrogenase (ENZ B)</strong> into each well. Cover plate tightly.</td>
</tr>
<tr>
<td>8.</td>
<td>Incubate at <strong>37°C for 15 minutes</strong>.</td>
</tr>
<tr>
<td>9.</td>
<td>Determine absorption at <strong>340 nm (OD&lt;sub&gt;SAMPLE&lt;/sub&gt;)</strong>.</td>
</tr>
<tr>
<td>10.</td>
<td>For analysis of obtained data see chapter 10 “evaluation of results”.</td>
</tr>
</tbody>
</table>
10. EVALUATION OF RESULTS

If the test is performed in strict compliance with the manufacturer’s instructions (i.e. with the exact volumes for standards, controls, and samples, and with correct sample treatment), standards, controls, and samples are equally diluted. Therefore, **no dilution factor is required for calculation of the results.**

Exception: If samples are diluted 1:2, the results must be multiplied by 2.

For calculation of results subtract OD values of the blank \((OD_{BLANK})\) from OD values after the addition of enzyme \((OD_{SAMPLE})\):

\[
\Delta OD = OD_{SAMPLE} - OD_{BLANK}
\]

To generate the standard curve, \(\Delta OD\) of the standards are plotted against the standard concentrations. With the obtained slope and y-intercept glutamate concentrations of the samples can be calculated:

\[
\text{glutamate [\mu mol/l]} = \frac{\Delta OD - \text{intercept}}{\text{slope}}
\]

**Controls**

Control samples should be analyzed with each run. Results generated from the analysis of control samples should be evaluated for acceptability using appropriate statistical methods. The results for the patient samples may not be valid if within the same assay one or more values of the quality control samples are outside the acceptable limits.

In the following an example of a calibration curve is given, do not use it for calculation of your results.
Expected values

Based on internal studies with serum samples of evidently healthy persons (n=24) a mean value of 144 μmol/l was estimated. The standard variation was 34 μmol/l.

\[
\text{mean value } \pm 2 \times \text{standard variation: } \quad 144 \pm 68 \; \mu\text{mol/l}
\]

\[
\text{normal range: } \quad 76 \text{ – } 212 \; \mu\text{mol/l}
\]

We recommend each laboratory to develop its own normal range. The values mentioned above are indicative only and can deviate from other published data.

11. PERFORMANCE CHARACTERISTICS

Precision and reproducibility

<table>
<thead>
<tr>
<th>Intra-Assay (n=6)</th>
<th>Glutamate [μmol/l]</th>
<th>Coefficient of variation (CV) [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>109.6</td>
<td>2.1</td>
</tr>
<tr>
<td>Sample 2</td>
<td>223.4</td>
<td>1.7</td>
</tr>
<tr>
<td>Sample</td>
<td>Glutamate [μmol/l]</td>
<td>Coefficient of variation (CV) [%]</td>
</tr>
<tr>
<td>--------</td>
<td>-------------------</td>
<td>----------------------------------</td>
</tr>
<tr>
<td>1</td>
<td>98.2</td>
<td>1.3</td>
</tr>
<tr>
<td>2</td>
<td>15.6</td>
<td>1.3</td>
</tr>
</tbody>
</table>

### Sensitivity

The sensitivity was set as $B_0 + 2SD$. The zero-standard was measured 20 times.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Glutamate mean value [OD]</th>
<th>2 x Standard variation (2 x SD)</th>
<th>Detection limit [μmol/l]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.02</td>
<td>12.6</td>
<td>5.9</td>
</tr>
</tbody>
</table>

### Recovery

One serum sample was spiked with different glutamate concentrations and measured in this assay. The analytical recovery rate was determined by the expected and measured glutamate levels. The expected levels were calculated as the sum of the measured glutamate concentration in the original sample and the spiked glutamate amount. The mean recovery rate for all concentrations was 100.5 % (n=6).

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>150.7</td>
<td>152.6</td>
<td>101.3</td>
</tr>
<tr>
<td>50</td>
<td>200.7</td>
<td>200.1</td>
<td>99.7</td>
</tr>
</tbody>
</table>

### Linearity

Linearity of the test was determined by diluting a spiked serum sample. The mean linearity was 95.5 %. 
### ELISA Glutamate

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>original</td>
<td>100.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1+1</td>
<td>50.4</td>
<td>49.6</td>
<td>98.4</td>
</tr>
<tr>
<td>1+3</td>
<td>25.2</td>
<td>23.3</td>
<td>92.5</td>
</tr>
</tbody>
</table>

### 12. LIMITATIONS

Hemolytic and lipemic samples may give erroneous results. Do not measure hemolytic and lipemic samples.

### 13. REFERENCES


### 14. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- All reagents in the test package are for research use only.
- Reagents should not be used after the date of expiry stated on the label.
• Single components with different lot numbers should not be mixed or exchanged.
• The guidelines for medical laboratories should be observed.
• Incubation time, incubation temperature, and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the test. Immundiagnostik AG can therefore not be held responsible for any damage resulting from wrong use.

*Used symbols:*
- **Temperature limitation**
- **Catalogue Number**
- **For research use only**
- **Contains sufficient for <n> tests**
- **Manufacturer**
- **Use by**
- **Lot number**