ATP Hexokinase FS* 
Diagnostic reagent for quantitative in vitro determination of ATP in blood and erythrocyte concentrates with Hexokinase method on photometric systems

Order Information
Cat. No. Kit size
1 6201 99 10 021 R1 5 x 20 mL + R2 1 x 25 mL
1 6200 99 10 065 3 x 3 mL Standard 100 µmol/dL

Summary [1,2]
ATP in blood and erythrocyte concentrates is an indirect indicator for the 24 h survival rate of erythrocytes [1]. Because of the low technical requirements, the measurement of ATP as an analytical check for the efficiency of erythrocyte concentrates is recommended [2].

Method
This method is traceable to the molar extinction coefficient.

Principle
Glucose + ATP HK Glucose-6-phosphate + ADP
Glucose-6-phosphate + NAD G6P-DH Gluconate-6-P + NADH + H+

Reagents
Components and Concentrations
R1: TRIS-buffer pH 7.8 0.1 mol/L
Mg2+ 4 mmol/L
Glucose 20 mmol/L
NAD 2.1 mmol/L
R2: Mg2+ pH 7.0 4 mmol/L
Hexokinase (HK) ≥ 7.5 kU/L
Glucose-6-phosphat-dehydrogenase ≥ 7.5 kU/L
(G6P-DH)

Storage instructions and reagent stability
The reagents and the standard are stable up to the end of the indicated month of expiry, if stored at 2 – 8 °C, protected from light and contamination is avoided. Do not freeze the reagents!

Warnings and precautions
1. The reagents contain sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
2. Reagent R2 contains biological material. Handle the product as potentially infectious according to universal precautions and good clinical laboratory practices.
3. In very rare cases, samples of patients with gammapathy might give falsified results. [6]
4. Sulfasalazine and sulfapyridine medication may lead to false results in patient samples. Blood collection must be done before drug administration.
5. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents. For diagnostic purposes, the results should always be assessed with the patient’s medical history, clinical examinations and other findings.
6. For professional use only!

Waste management
Please refer to local legal requirements.

Reagent preparation
The reagent and the standard are ready to use.

Materials required but not provided
Trichloroacetic acid 12% (w/v)
General laboratory equipment

Sample preparation
Pipette 1.0 mL blood or erythrocyte concentrate and 1.0 mL trichloroacetic acid 10 - 12% (w/v) into a centrifuge tube, mix well and put into an ice bath for approx. 5 min. Centrifuge the sample solution 5 - 10 min at approx. 3000 g. After centrifugation use 250 µL of the clear supernatant directly and without any waiting time in the assay.

Use the ATP standard without sample preparation directly in the assay. When using ATP standard for calibration patient results have to be multiplied by 2.

Note:
ATP in samples is unstable. The ATP content of blood collected in heparin or EDTA shows a decrease of 80 % within 24 h, if stored at 2 – 8 °C [3]. Storage of samples mixed with trichloroacetic acid at - 20 °C gives false results, too. Due to this samples clarified with trichloroacetic acid must be used directly in the assay.

Assay procedure
Wavelength 340 nm, Hg 365 nm, Hg 334 nm
Optical path 1 cm
Temperature 20 - 25 °C
Measurement Against air or water

Calculation
Multiply ΔA by the corresponding factor F from table below in order to calculate the ATP concentration:

| Wavelength | 340 nm | 412.70 | 206.35 |
| Hg 334 nm | 420.71 | 210.36 |
| Hg 365 nm | 764.71 | 382.35 |

F = (V x f x 100) / (ε x v x d) [µmol/dL]

V = Total volume in cuvette [µL]
f = Dilution factor of sample preparation = 2.0
D = Light path [cm] = 1.00
v = Sample volume [µL] = 250
ε = Ext. coefficient NADH [l x cm⁻¹ x mmol⁻¹] = 6.3 at 340 nm = 3.4 at 365 nm = 6.18 at 334 nm

c = Ext. coefficient NADH [l x cm⁻¹ x mmol⁻¹] = 6.3 at 340 nm = 3.4 at 365 nm = 6.18 at 334 nm

Quality control
For internal control of precision and accuracy DiaSys ATP Standard (Cat.-No. 1 6200 99 10 065, 3 x 3 mL) is recommended. Each laboratory should establish corrective action in case of deviations in control recovery.
Performance characteristics

Measuring range
The test has been developed to determine ATP concentrations within a measuring range from 37 – 370 µmol/dL measured at 365 nm and 20 – 400 µmol/dL measured at 334/340 nm. If values exceed this range, sample volume has to be decreased to 125 µL, and factor F must be recalculated with the new sample volume.

Interferences
Because of the preparation with trichloroacetic acid no interference was observed by lipemia, hemoglobin, ascorbic acid up to 30 mg/dL and bilirubin up to 60 mg/dL. For further information on interfering substances refer to Young DS [5].

Limit of detection
The lower limit of detection is 1.5 µmol/dL. Limit of detection corresponds with the smallest ATP concentration differentiating from zero. Sensitivity is calculated out of three standard deviations from 20 replicates of a zero sample.

Precision
For measurement erythrocyte concentrates and spiked serum samples were used.

<table>
<thead>
<tr>
<th>Intra-assay</th>
<th>Mean [µmol/dL]</th>
<th>SD [µmol/dL]</th>
<th>CV [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>9.3</td>
<td>0.12</td>
<td>1.31</td>
</tr>
<tr>
<td>Sample 2</td>
<td>34.6</td>
<td>0.29</td>
<td>0.82</td>
</tr>
<tr>
<td>Sample 3</td>
<td>58.5</td>
<td>1.07</td>
<td>1.82</td>
</tr>
</tbody>
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<tbody>
<tr>
<td>Sample 1</td>
<td>43.7</td>
<td>1.56</td>
<td>3.57</td>
</tr>
<tr>
<td>Sample 2</td>
<td>88.5</td>
<td>3.19</td>
<td>3.60</td>
</tr>
<tr>
<td>Sample 3</td>
<td>182.8</td>
<td>6.48</td>
<td>3.55</td>
</tr>
</tbody>
</table>

Recovery rate
Recovery rate of spiked erythrocyte concentrates is approx. 92%.

Reference range [4]
Blood ATP 38 - 62 µmol/dL
Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

Literature
3. Data on file at DiaSys Diagnostic Systems GmbH

Manufacturer
DiaSys Diagnostic Systems GmbH
Alte Strasse 9 65558 Holzheim Germany