ATP Hexokinase FS*

Order Information

Cat. No.	Kit size				
1 6201 99 10 021	R1	5 x 20 mL	+	R2	1 x 25 mL

Intended Use

Diagnostic reagent for quantitative in vitro determination of ATP in blood and erythrocyte concentrates on photometric systems.

Summary

The ATP content in blood and erythrocyte concentrates provides information on the viability of the erythrocytes. It has been shown that the ATP content in erythrocytes correlates well with 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 concentrate is recommended [2].

Method

This method is traceable to the molar extinction coefficient.

$$\text{Glucose + ATP} \xrightarrow{\text{HK}} \text{Glucose-6-phosphate + ADP}$$

$$\text{Glucose-6-phosphate + NAD}^+ \xrightarrow{\text{G6P-DH}} \text{Gluconate-6-P + NADH + H}^+$$

Reagents

Components and Concentrations

R1:	TRIS	pH 7.8	0.1 mol/L
	Mg^{2+}		4 mmol/L
	Glucose		20 mmol/L
	NAD		2.1 mmol/L
R2:	Mg^{2+}	pH 7.0	4 mmol/L
	Hexokinase (HK)		≥ 7.5 kU/L
	Glucose-6-phosphat-dehydrogenase (G6P-DH)		≥ 7.5 kU/L
Standard:			100 µmol/dL

Storage and Stability

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

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 2 contains animal material. Handle the product as potentially infectious according to universal precautions and good laboratory practice.
3. In very rare cases, samples of patients with gammopathy might give falsified results. [3]
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

Refer to local legal requirements.

Reagent Preparation

The reagent and the standard are ready to use.

Materials Required

Trichloroacetic acid 10 – 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 [4]. 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.

Discard contaminated specimens.

Assay Procedure

Applications for automated systems are available on request.

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

	Blank	Sample or standard
Sample or standard	-	250 µL
Dist. Water	250 µL	-
Reagent 1	2400 µL	2400 µL
Mix, incubate for 3 – 5 min. at 25°C, read absorbance (A1), then add:		
Reagent 2	600 µL	600 µL
Mix, incubate for approx. 15 min. at 25°C read absorbance (A2) within 30 min.		

Calculation

$\Delta A = [A2 - A1]_{Sample/Standard} - [A2 - A1]_{Blank}$

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

	With sample preparation [µmol/dL]	Without sample preparation [µmol/dL]
340 nm	412.70	206.35
Hg 334 nm	420.71	210.36
Hg 365 nm	764.71	382.35

$F = (V \times f \times 100) / (\varepsilon \times v \times d)$ [µmol/dL]

V	= Total volume in cuvette [µL]	= 3250
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

Quality Control

DiaSys ATP Standard FS is recommended for internal control of precision and accuracy. Each laboratory should establish corrective action in case of deviations in control recovery.

	Cat. No.	Kit size
ATP Standard FS	1 6200 99 10 065	3 x 3 mL

Performance Characteristics

Exemplary data mentioned below may slightly differ in case of deviating measurement conditions.

Measuring range up to 37 – 370 µmol/dL measured at 365 nm and 20 – 400 µmol/dL measured at 334/340 nm. When values exceed this range, sample volume has to be decreased to 125 µL, and factor F must be recalculated with the new sample volume.	
Limit of detection**	1.5 µmol/dL

Interfering substance	**Interferences ≤ 10% up to**
Ascorbic acid	30 mg/dL
Bilirubin	60 mg/dL
Hemoglobin	30 mg/dL
Lipemia	30 mg/dL
For further information on interfering substances refer to Young DS. [5]	

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

Within run (n=20)	**Sample 1**	**Sample 2**	**Sample 3**
Mean [µmol/dL]	9.8	34.6	58.5
CV [%]	1.24	0.85	1.87
Between day (n=20)	**Sample 1**	**Sample 2**	**Sample 3**
Mean [µmol/dL]	43.7	88.5	183
CV [%]	3.66	3.69	3.64

** lowest measurable concentration which can be distinguished from zero; mean + 3 SD (n = 20) of an analyte free specimen.

Recovery rate

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

Reference Range

Blood ATP [6] 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

1. Högmann CF, de Verdier CH, Ericson A: Studies on the mechanism of human red cell loss of viability during storage at 4°C in vitro. 1. Cell shape and total adenylate concentration as determinant factors for posttransfusion survival. Vox Sang 48: 257-268, 1985.
2. Heiden M., Seitz R: Zulassung von Blutkomponenten zur Transfusion. Bundesgesundheitsbl-Gesundheitsforsch-Gesundheitsschutz. 42: 150-155 Springer Verlag, 1999.
3. Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. ClinChemLabMed 2007;45(9):1240-1243.
4. Data on file at DiaSys Diagnostic Systems GmbH.
5. Young DS. Effects of Drugs on Clinical Laboratory Tests. 5th ed. Volume 1 and 2. Washington, DC: The American Assocation for Clinical Chemistry Press 2000.
6. Dennemann H: Enzymatische Bestimmung von Adenosin-triphosphat in Vollblut. Z Gesamte Experimentelle Medizin 134:335, 1961.

DiaSys Diagnostic Systems GmbH
Alte Strasse 9 65558 Holzheim Germany
www.diasys-diagnostics.com

* Fluid Stable