PYRUVATE

(Quantitative Enzymatic UV–Test)

Cat.No.			Package Size
	R1a	R1b	R2
180 000	2 x 50 mL	1 x 5.0 mL	1 x 5.0 mL
	Standard 1 x 20 mL		

METHOD / TESTPRINCIPLE

Enzymatic. UV-Test .
Endpoint determination of pyruvate in blood :

In the presence of an excess of NADH pyruvate is converted to lactate.
The reduction of the absorbance = ΔA ,at 340 nm, due to the oxidation of NADH to NAD^+ , is a measure of the amount of pyruvate originally present :

Pyruvate + NADH + H^+ $\xrightarrow{LDH}$ L-Lactate + NAD^+

LDH = Lactatedehydrogenase

REAGENTS COMPOSITION

R1a :	(Tris buffer, pH 7.20)	1.50 mol/L
R1b :	NADH	10.0 mmol/L
Start-Reagent R2 :	LDH	1.50 kU/mL
Standardsolution	Pyruvate	4.00 mg/dL

Additional Reagent *(not provided with the kit)*

Cat.No.502 001 (2 x 500ml) Deproteinization Reagent (Perchloric Acid 0.6m)

PRECAUTIONS

- For in vitro diagnostic use only.
- Reagents contain < 0,95 g/ L sodium azide. Avoid contact with skin and/or mucous membranes
- Avoid contamination by using clean laboratory material (pipettes, plastic vials for analyzers, ...).
- Discard cloudy i.e. deteriorated reagent.

STABILITY OF REAGENTS

When stored at 2-8° C and protected from light, the reagents are stable until the expiry date stated on the labels.

PREPARATION AND STABILITY OF WORKING REAGENTS

Reagents (R1a ,R1b and reagent R2) are ready for use

SAMPLE PREPARATION

Pipet 2,0 mL of **freshly drawn blood** into a centrifugation tube containing 4 mL of cold **0.6 m perchloric acid**. Vortex for about 30 seconds.
Keep the blood precipitate mixture for about 5 min in the cold to assure complete protein precipitation. Centrifuge 10 min at approximately 1500 x g. The protein free supernatant is ready for use.
The **Standardsolution** has to be diluted with perchloric acid, in the same ratio as the sample.

MANUAL PROCEDURE

Wavelength : 340 nm (334-365)
Temperature : 30°C, 37°C
Cuvette : 1 cm light path
(Measure against water)

	Supernatant Sample	Diluted Standard
Buffer Reagent R1a	1.00 mL	1.00 mL
Supernatant Sample	2.00 mL	-
Diluted Standard	-	2.00 mL
Mix and add **Reagent R1b**	50 µL	50 µL
Mix and incubate for approx. 5 min . Pour into cuvette, measure initial absorbance A_1.		
Start-Reagent R2	50 µL	50 µL
Mix, incubate for approx. 5 min and measure absorbance A_2		

$\Delta A = A_2 - A_1$ Sample and Standard

CALCULATION

- **with Factor :**
  Pyruvate (mg/dL) = ΔA_{340} x 6,37

- **with Standard :**
  Pyruvate (mg/dL) = ΔA_{sample} x 4.00 / $\Delta A_{Standard}$

REFERENCE VALUES (37°C) :

0,3 – 0,7 mg/dL

Note: It is recommended for each laboratory to establish and maintain its own reference values. The given data are only an indication.

QUALITY CONTROL

For quality control use adequate control materials, available from Greiner .

Greiner Diagnostic GmbH - Unter Gereuth 10 – D-79353 Bahlingen – Germany
www.greiner-diagnostic.com