# PHI

**Kinetic UV–Test for the determination of Phosphohexose Isomerase in serum and plasma**

| Cat. No | Package Size |
|---|---|
| 178 080 | R1 (Buffer) 4 x 6 ml / R2 (Lyophilisate) for 4 x 6 ml |

## Method / Testprinciple

Kinetic UV-Test according to Bueding and Mackinnon:

Fructose-6-phosphate $\xrightarrow{PHI}$ Glucose-6-phosphate

Glucose-6-phosphate + $NAD^+$ $\xrightarrow{G6P\text{-}DH}$ 6-Phosphogluconate + $NADH_2^+$

## Reagents

**Constituents ( concentrations in the test)**

| | | |
|---|---|---|
| **R1:** | TRIS-buffer (pH 8.5) | 100 mmol/l |
| **R2:** | Fructose-6-phosphate | 3 mmol/l |
| | Nicotinamide-adenin-dinucleotide (NAD) | 3 mmol/l |
| | Glucose-6-phosphate-dehydrogenase (G6P-DH) | 1 U/ml |

## Storage and stability

The reagents are stable up to the expiry date printed on the labels, when stored at 2-8° C and protected from light.
After reconstitution the reagent solution is stable at least for 2 weeks at 2 – 8°C, when contamination is avoided.

**Precautions**

-For in vitro diagnostic use only.
-The reagent contains < 0,95 g/L sodium azide.
Avoid contact with skin and/or mucous membranes.

**Waste**

Treat according to the local legal regulations

**Preparation**

***Reagent R :***

Reconstitute 1 bottle of lyophilisate R2 („for 6 ml") with 1 bottle of buffer R1.
R is after 15 min ready for use.

*Stability in tightly closed bottle and without contamination: :*

4 days at 2 – 8 °C
1 day at 18 – 25 °C

## Samples

Serum, plasma, liquor

*Stability of PHI in serum* *2 weeks at 2 - 8 °C*

*3 months ati < - 25 °C*
***(Freeze only once!)***

## Procedure

| | |
|---|---|
| Wavelength | 340 nm, Hg 334 nm, Hg 365 nm |
| Cuvette | 1 cm |
| Temperature | 37°C (25 °C) |
| Measure | against air (increasing absorbance) |

| **Pipette into cuvettes** | | |
|---|---|---|
| | **Semi-Micro** | **Micro** |
| **Reagent R** | 1000 µl | 500 µl |
| **Sample** | 50 µl | 20 µl |
| Mix well, incubate 1 min at constant temperature, read absorbance A and immediately start stop watch. After exactly 1, 2 and 3 min read again. Calculate ΔA/min | | |

## Calculation

**ΔA/min x Factor = PHI-Activity [U/l]**

| **Factors for 37 °C (25 °C)** | | | | |
|---|---|---|---|---|
| **Wavelength** | **Semi-Micro** | | **Micro** | |
| **Hg 334 nm** | 6150 | (3398) | 7615 | (4207) |
| **340 nm** | 6033 | (3333) | 7470 | (4127) |
| **Hg 365 nm** | 1117 | (6176) | 13841 | (7647) |

***Applications for automated systems are available on request***

## Calibration and Quality Control

For the calibration of automated analyzers Greiner multicalibrator Unical-M is recommended, for quality control use Greiner normal and abnormal controls Unitrol-I and Unitrol-II respectively the PHI special control



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