UREA UV LR liquid reagent

REF **E4805100** R1 5x80 ml/ R2 1x100 ml
E4800550 R1 5x40 ml/ R2 1x50 ml
E480340130 R1 3x40 ml/ R2 1x30 ml

CE IVD For in vitro medical device

Use
Kit for measurement of urea in serum, plasma and urine. Kinetic UV method Urease-GLDH

Summary
Urea Increase in serum can be the result of kidney dysfunction or urinary tract obstruction.

Principle
Fixed time analysis. Urea is hydrolysed, in the presence of water, in urease to produce carbon dioxide. The ammonia produced, in the first reaction, combines with alpha-ketoglutarate and NADH which, in the presence of glutamate-dehydrogenase, forms glutamate and NAD+. The decrease in absorbance at 340 nm due to depletion of NADH is proportional to the urea concentration in the sample.

Reagents

R1	Goods buffer pH 7.6	130.0 mmol/l
	ADP	1.2 mmol/l
	Urease	≥ 8000 U/l
	GLDH	≥ 1500 U/l
R2	Goods buffer pH 10.2	100.0 mmol/l
	alpha-ketoglutarate	65.0 mmol/l
	NADH	1.20 mmol/l

Preparation of Reagents
Reagents are liquid and ready to use. About using as monoreagent ("sample-starter" procedure) add to every 4 ml of R1 reagent, 1 ml of R2 reagent.
Keep out the reagents from refrigerator only for the use and recap them immediately.

Storage and stability
- Store the kit at 2-8°C
- After opening, the vials R1, R2 are stable 90 days if recapped immediately and protected from contamination, evaporation, direct light, and stored at the correct temperature.
- Working solution stability (R1+ R2): 20 days at 2-8°C.

Precaution in Use
The product is not classified as dangerous (DLg. N. 285 art. 28 l. n. 128/1998.
However the reagent should be handled with care, according to good laboratory practice.
Caution: the reagents contain Sodium Azide (0.095%) as preservative. Avoid swallowing and contacting with skin, eyes and mucous membranes.
In case of contact with eyes rinse immediately with plenty of water and seek medical advice.

Waste Management
Please refer to the local legal requirements.

Specimen collection and preparation
- Serum or plasma.
- Diluted urine 1:20.
- Do not use samples with haemolysis.
- Do not use ammonia-heparinate and fluorides as anticoagulants.
- The urea in the serum is stable up to 3 days if stand at 2-8°C or 3 months at -20°C.

Note
- The kit, according to this method, must be used in manual procedures. About automatic using follow specific applications.
- Avoid direct light, contamination and evaporation.
- The volumes in the procedure can be changed proportionally.
- In case of complaint or quality control request, refer to the lot number on the package or the lot number on the singles vials.

Procedure

Wavelength	λ: 340 (334-365) nm
Working temperature	37°C
Optical path	1 cm
Reaction	"fixed time" (in decreasing)

Bring the reagents at 15 -25°C before using them.

- Monoreagent Procedure "sample starter"

	BLANK	STD	SAMPLE
Working reagent	1000 µl	1000 µl	1000 µl
Distilled Water	10 µl	--	--
Sample	--	--	10 µl
Standard	--	10 µl	--

Mix, then incubate at 37°C. Measure the absorbance values of first reading after 30" from sample adding (E1C) and standard (E1STD). Read a second time after 60" (E2C), (E2STD).

- Bireagent Procedure "substrate starter"

	BLANK	STD	SAMPLE
Reagent R1	800 µl	800 µl	800 µl
Distilled Water	10 µl	--	--
Sample	--	--	10 µl
Standard	--	10 µl	--

Mix, incubate at 37°C for 1' and then add:

	BLANK	STD	SAMPLE
Reagent R2	200 µl	200 µl	200 µl

Mix, then incubate at 37°C. Measure the absorbance values of first reading after 30" from sample adding (E1C) and standard (E1STD). Read a second time after 60" (E2C), (E2STD).

Calculation
Urea [mg/dl] o [mmol/l] =
(E2C - E1C) / (E2STD - E1STD) x Conc. STD
Diluted urines: multiply the result for diluting factor.

Conversion Factor
Urea [mg/dl] x 0.1665 = Urea [mmol/l]

Reference Values

Serum - plasma	10 - 50 mg/dl (1.67 - 8.32 mmol/l)
Urine	20 - 35 g/24h (333 - 583 mmol/24h)

Reference values are considered indicative since each laboratory should establish reference ranges for its own patient population. The analytical results should be evaluated with other information coming from patient's clinical history.

ANALYTICAL PERFORMANCES

Linearity
Reaction is linear up to a concentration of 200 mg/dl (33.3 mmol/l) with a range of 4.9-200 mg/dl (0.81-33.3 mmol/l). Samples with values exceeding 200 mg/dl must be diluted with saline solution. Multiply, then, the result for diluting factor.

"Intra-Assay" precision (within-Run)
Determined on 20 samples for each control (N-H) (Normal-High). Results :

MEANS [mg/dl]	N = 40.40	H = 150.95
S. D.	N = 1.50	H = 2.18
C.V.%	N = 3.70	H = 1.44

"Inter-Assay" precision (between-Run)
Determined on 20 samples for each control (N-H) (Normal – High). Results :

MEANS [mg/dl]	N = 40.63	H = 152.05
S. D.	N = 1.20	H = 2.46
C.V.%	N = 2.95	H = 1.62

Analytical Sensitivity
The test sensitivity in terms of detection limit is 3.0 mg/dl (0.50 mmol/l).

Interferences
No interference was observed by presence of:

Bilirubin	≤ 20 mg/dl.
Triglycerides	≤1000 mg/dl.
Hemoglobin	≤ 100 mg/dl.

Correlation
A study based comparing this method with a similar method on 20 samples has given a correlating factor
r = 0.99
y = 1.147x + 0.061

Quality Controls
It's necessary, each time the kit is used, to make the quality controls and to check that values obtained are within the acceptance range provided in the insert each laboratory should establish its own mean and standard deviation and adopt a quality control program to monitor laboratory testing.

Bibliography
Talke H, Schubert GE: Klin Wchers., (1965), 43, 174.
Kaplan LA, Pesce AJ: "Clinical Chemistry", Mosby Ed. (1996).

Symbols

CE	CE Mark (98/79 CE regulation)
IVD	in vitro medical device
LOT	Batch Code
	Use by
	Storage temperature limits
	Read instruction for use
	Gesan Production srl