ENZYMATIC CREATININE

Diagnostic reagent for determination of Creatinine concentration.
Liquid. Dual reagents. Store at +2/+8°C. For in Vitro Diagnostic Use (IVD). **Do not freeze.**

Ref No	Package	Ref No	Package	Ref No	Package	Ref No	Package
A2091N	500 mL	D2090	900 mL	L2090	600 mL	M4091	400 mL
A2092N	200 mL	D2091	500 mL	L2091	400 mL	M4092	560 mL
A2093N	100 mL	ER2090	440 mL	L2092	240 mL	PL2090	240 mL
A2094N	200 mL	ER2091	308 mL	MD090	450 mL	PL2091	150 mL
BB050	220 mL	HN090	600 mL	MD091	225 mL	RD2090	400 mL
BY2090	700 mL	HN091	400 mL	M2090	600 mL	RD2091	320 mL
BY2091	500 mL	K2091	320 mL	M2091	400 mL	S2092	200 mL
BZ2050	360 mL	LB090	240 mL	M3090	320 mL	TB2090	400 mL
DM2090	333 mL	LM065	240 mL	M3091	120 mL	TB2091	200 mL
		LM066	240 mL			8A2090	700 mL
						8A2091	500 mL

Changes made in the instructions for use are marked as grey.

INTENDED USE

The test is applied for the quantitative enzymatic determination of creatinine in serum, plasma and urine.

GENERAL INFORMATION

Some of the free creatine in muscle is spontaneously and irreversibly converted into creatinine, the anhydride waste product. Thus, the amount of creatinine produced each day in an individual is fairly constant and correlates with muscle mass. In a healthy individual, the blood concentration of creatinine is also fairly constant, although it can be influenced by diet. Creatinine is present in all body fluids and secretions and is freely filtered through the glomeruli. Although it is largely not reabsorbed by the renal tubules, there is a small but significant tubular secretion as well as concentration-dependent losses in the intestine.[1] Creatinine production also decreases as the concentration of circulating creatinine increases. Several mechanisms have been proposed for this, including feedback inhibition of creatinine production, recycling of creatinine to creatine and conversion to other metabolites.[2-4] Serum creatinine concentration is a product of the rate of release from muscle into circulation and the rate of removal. Creatinine measurement is easy and inexpensive. However, its production is influenced by age, sex, race, muscle mass and diet, as well as various preanalytical and analytical effects.[5,6] An important issue is that the serum creatinine level may remain within the reference range until renal function is substantially lost.[5]

Glomerular filtration rate (GFR) is most commonly assessed using methods based on measurement of serum creatinine. Creatinine is freely filtered through the glomeruli and its concentration is inversely related to GFR, i.e. for the same creatinine production, halving the GFR leads to approximately doubling the serum creatinine concentration.[1] A small (but significant) and variable proportion (≈7-10%) of the amount of creatinine seen in urine is due to tubular secretion.[7] However, this amount increases in the presence of renal impairment and is inhibited by some drugs (e.g. cimetidine[7] and trimethoprim).[8]

Serum creatinine measurement is used in the diagnosis and monitoring of acute and chronic renal disease, measurement of glomerular filtration rate (GFR) or to assess the condition of renal dialysis patients. Urine creatinine analysis is used to calculate creatinine clearance, to verify completion of 24-hour collections, or to provide a reference amount for other analytes, such as calculation of the albumin/creatinine ratio. Urine creatinine analysis is used to calculate creatinine clearance, as well as to verify completion of a 24-hour urine sample collection or to provide a reference quantity for other analytes, such as calculation of the albumin/creatinine ratio. Measurement of creatinine in serum is also used in the diagnosis, treatment and follow-up of some renal diseases, in monitoring the condition of renal dialysis patients, and in the interpretation of some other analytes after their measurement and ratio to creatinine.[1,9]

Measurement of serum creatinine is crucial in the detection of acute kidney injury (AKI); the definition and classification of AKI is predominantly based on changes in serum creatinine concentration over time.[1] Kidney Disease Improving Global Outcomes (KDIGO) defines ABH as an increase in serum creatinine of ≥0.3 mg/dL (≥26.5 mmol/L) within 48 hours or an increase in serum creatinine of ≥1.5 times higher than baseline within the previous 7 days.[10] The KDIGO guidelines also recommend the use of serum creatinine and the GFR estimation equation (eGFR) in the initial evaluation of chronic kidney disease (CKD). Categorized GFR values (G1 to G5 classification) and albuminuria values (A1 to A3 classification) categorized according to the albumin/creatinine ratio (ACR) are used in the prognosis of CKD, prediction of cardiovascular and all-cause mortality, diagnosis and follow-up of end-stage renal failure, and evaluation of CKD progression and acute kidney injury. In addition to ACR values, protein creatinine ratio (PCR) is also used in the evaluation of diseases such as nephrotic syndrome. As an important note, serum

creatinine is not exactly an ideal marker for the calculation of GFR; therefore, creatinine-derived equations such as Modification of Diet in Renal Disease (MDRD) and GFR-EPIcrea are not suitable for use in patients with diseases such as AKI, where serum creatinine concentrations can change rapidly.[1]

TEST PRINCIPLE

Enzymatic method

Creatinine in the sample is hydrolyzed to creatine by creatinase. Creatine is then hydrolyzed to sarcosine and urea by creatinase. The sarcosine formed by this reaction is oxidized to glycine and formaldehyde by sarcosine oxidase, and at this time hydrogen peroxide is released. Hydrogen peroxide reacts with 4-aminoantipyrine and ESPMT catalyzed by peroxidase to form the chromogenic quinonimine dye.

The enzymatic method utilizes a two-reagent system that eliminates the interaction by endogenous creatine and ascorbic acid.

$$\text{Creatinine} + H_2O \xrightarrow{\text{Creatinine aminohydrolase}} \text{Creatine}$$

$$\text{Creatine} + H_2O \xrightarrow{\text{Creatinine aminohydrolase}} \text{Sarcosine} + \text{Urea}$$

$$\text{Sarcosine} + H_2O + O_2 \xrightarrow{\text{Sarcosine oxidase}} \text{Glycine} + HCHO + H_2O_2$$

$$2H_2O_2 + \text{4-aminoantipyrine} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine Dye} + 4H_2O$$

The absorbance of the color produced at 548 nm is proportional to the creatinine concentration in the sample.

REAGENT COMPONENTS

Reagent 1:

Good Buffer	: 25 mmol/L
Creatine amidinohydrolase	: > 20 KU/L
Sarcosine oxidase	: > 6 KU/L
Ascorbate oxidase	: > 5 KU/L
ESPMT	: >120 mg/L

Reagent 2:

Good Buffer	: 90 mmol/L
Creatinine amidohydrolase	: > 230 KU/L
Peroxidase	: > 4 KU/L
4-aminoantipyrine	: >500 mg/L
ESPMT	

REAGENT PREPARATION

Reagents are ready for use.

Annotation:

- The potential interference from ascorbic acid is prevented by the inclusion of ascorbate oxidase.[11]

REAGENT STABILITY AND STORAGE

Reagents are stable at +2/+8˚C till the expiration date stated on the label which is only for closed vials.

Once opened vials are stable for 30 days at +2/+8°C in optimum conditions. On board stability is strongly related to auto analyzers' cooling specification and carry-over values.

Reagent stability and storage data have been verified by using Clinical and Laboratory Standards Institute (CLSI) EP25-A protocol.[12]

SAMPLE REQUIREMENTS

Serum, plasma and urine are collected by standard procedures. Multiple sample freezing and thawing should be avoided.

Creatinine activity stability in serum:

7 days at +20/+25ºC
7 days at +2/+8ºC
3 months at -20°C

Creatinine activity stability in urine (without preservatives):

2 days at +20/+25ºC
6 days at +2/+8ºC
6 months at -20°C

Creatinine activity stability in urine (with preservatives):

3 days at +20/+25ºC
8 days at +2/+8ºC
3 weeks at -20°C

Annotation:

- Acidified urine is not suitable for creatinine determination.
- The concentration of creatinine in the blood increases after meals containing cooked meat or fish because creatine is converted to creatinine. Ideally, blood for serum creatinine measurement should be taken in the fasting state.[13-19] Although the effect depends on the amount and type of meat consumed and the time of sampling, it can increase the creatinine concentration by 25%,[18] with a similar reduction in creatinine-based GFR estimates.[1]

CALIBRATION AND QUALITY CONTROL

Calibration: The assay requires the use of Creatinine Enzymatic Calibrator Set.

Creatinine Enzymatic Calibrator Set
Ref.No: A237S

Calibration stability is 30 days. Calibration stability depends on the application characteristics and cooling capacity of the autoanalyzer used.

Control: Commercially available control material with established values determined by this method can be used. We recommend:

Ref.No: A3910

Ref.No: A3912 (For Olympus AU series.)
Ref.No: A3913 (For BS series.)
Ref.No: A3914 (For Erba.)

Arcon P Level 2 Control- Lyophilized
Ref.No: A3920
Ref.No: A3922 (For Olympus AU series.)
Ref.No: A3923 (For BS series.)
Ref.No: A3924 (For Erba.)

At least two level controls must be run once in every 24 hours. Each laboratory should determine its own quality control scheme and procedures. If quality control results are not within acceptable limits, calibration is required.

REFERENCE INTERVALS / MEDICAL DECISION LEVELS

Serum samples:

Men	: 0.70 - 1.20 mg/dL
Women	: 0.60 - 1.10 mg/dL
Children	
0 – < 15 days	: 0.42 - 1.05 mg/dL
15 days – <1 year	: 0.45 – 0.70 mg/dL
1 year – <4 years	: 0.50 – 0.74 mg/dL
4 years – <7 years	: 0.65 – 0.80 mg/dL
7 years – <12 years	: 0.70 – 0.88 mg/dL
12 years – <15 years	: 0.70 – 0.93 mg/dL
15 years – <17 years	: 0.72 – 1.05 mg/dL

Urine, random:

Men	< 40 years	: 24 - 392 mg/dL
Men	≥ 40 years	: 22 - 328 mg/dL
Women	< 40 years	: 16 - 327 mg/dL
Women	≥ 40 years	: 15 - 278 mg/dL

24 hour urine:

Men	:1040-2350 mg/24hour (9000 - 21000 µmol/24hour)
Women	:740-1570 mg/24hour (7000 - 14000 µmol/24hour)

For 24-hour urine excretion, to convert results from mg/dL to mg/24-hour;

24 h urine = [(V x c) / 100] mg/24-hour (or day)

V = 24 hour urine volume
c = analyte concentration (mg/dL)

Annotation:

- Serum creatinine reference intervals depend on the method.[11]
- Since most creatine is found in muscle, the amount of creatinine in a person reflects muscle mass. This is why women have lower serum creatinine concentrations than men, and children and infants have lower serum creatinine concentrations than adults.[11]
- Reference intervals in older people are, on average, similar to those of young adults, despite the decline in renal function that occurs with aging.[11]

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary, determine its own reference range.

Reference interval has been verified by using CLSI EP28-A3c protocol.[20]

Unit Conversion:

Serum
mg/dL x 88.4= µmol/L
µmol/L x 0.001 = mmol/L

Urine
mg/dL x 0.0884 = mmol/L

PERFORMANCE CHARACTERISTICS

Measuring Interval
According to CLSI EP34-ED1:2018, "Measuring Interval" refers to the interval where the analyte concentration is measured with intended accuracy in terms of medical and laboratory requirements without dilution, concentrating or any kind of pre-treatment that is between the analyte's lower limit of quantitation (LLoQ) and upper limit of quantitation (ULoQ).[21]

The determined analytic measuring interval for Enzymatic Creatinine is 0.04 – 25 mg/dL.

Detection Capability

Limit of Detection (LoD): 0.02 mg/dL

Limit of Quantitation (LoQ): 0.04 mg/dL

Note: LoQ values are based on Coefficient of Variation Percentage (CV) ≤ 20%.

LoD and LoQ values have been verified by using CLSI EP17-A2:2012 protocol.[22]

Linearity

This method shows measurement linearity in the activities up to 25 mg/dL. Autoanaylzer's auto-dilution system can be used if the concentrations have higher values. See device manual for further information.

For the manual dilution procedure, dilute the sample 1:5 using 0.90% isotonic. After this process, multiply the result of the reworked sample by the dilution factor. Do not report the sample result after dilution if it is marked as lower than the linear lower limit. Rerun with a suitable dilution.

Linearity Studies data have been verified by using CLSI EP06-A:2003 protocol.[23]

Precision

Running system has been developed according to 20x2x2 "The Single Site" protocol. Repeatability and Within-Laboratory Precision/Within-Device values have been obtained according to the running results.

According to the protocol in use, 2 separate runs per day have been made for 20 days (no obligation for being consecutive days). This protocol has been applied to each low and high samples separately and 80 results have been obtained for each one. Statistically, the results have been obtained using 2-factor Nested-ANOVA model.[24]
Repeatability (Within Run) and Repeatability (Day to Day) SD (standard deviation) and CV% values of Enzymatic Creatinine have been given in the table 1 and 2 respectively.

Table 1. Enzymatic Creatinine Repeatability (Within Run) results obtained from samples in two different concentrations

Mean Concentration	SD	CV%	n
0.66 mg/dL	0.01	1.51	80
5.14 mg/dL	0.02	0.39	80

Note: This working system has been named "Within-Run Precision" in the previous CLSI - EP05-A2 manual.[25]

Table 2. Enzymatic Creatinine Repeatability (Day to Day) results obtained from samples in two different concentrations

Mean Concentration	SD	CV%	n
0.66 mg/dL	0.02	3.33	80
5.14 mg/dL	0.12	2.34	80

Note: This working system has been named "Total Precision" in the previous CLSI - EP05-A2 manual.[25]

Method Comparison

As a result of the statistical evaluation of the method comparison data:

Passing-Bablok equation:[26]
y= 1.221x – 0.251 mg/dL
r=0.9986

Interference

Endogenous interferant and analyte concentrations that have been used in the Enzymatic Creatinine scanning tests has been determined according to "CLSI EP37-ED1:2018" and "CLSI EP07-ED3:2018" manuals.[27,28]

The total acceptable error rate, which is going to be used to detect whether the observed differential value obtained from Enzymatic Creatinine interference scanning test is appropriate, is determined as ±10%.[29]

In Enzymatic Creatinine test results, no significant interaction has been observed in the determined endogenous interferant and analyte concentrations or between interferants and analyte.

Interferant-Concentration	Enzymatic Creatinine Target (mg/dL)	N*	Observed Recovery %
Hemoglobin 125 mg/dL	0.86	3	91
Bilirubin 12.15 mg/dL	0.78	3	109
Lipemia 1965 mg/dL	0.82	3	101

** Total acceptable error rate determined as interference limit and repeatability (within run) pre-detected for the related method were used for the calculations of how many times the control and test samples prepared as a serum pool are going to be run repetitively. In the calculations, the accepted error rate for type 1 (α error) was 5% and for type 2 (β error) was 10% (90% power).*[28]

Annotation:

- Non-hemolysed samples should be preferred.

It should be noted that endogenous interferants, as well as various medicines and metabolites, anticoagulants (e.g. Heparin, EDTA, citrate, oxalate) and preservatives (e.g. sodium floride, iodoacetate, hydrochloride acide) such as additives, materials that may contact with samples during collection and processing (serum separator devices, sample collection containers and contents, catheters, catheter wash solutions, skin disinfectants, hand cleaners and lotions, glass washing detergents, powder gloves), dietary substances known to affect some specific tests (caffeine, beta-carotene, poppy seeds, etc.), or some substances present in a sample that cause foreign proteins (heterophilic antibodies, etc.), autoimmune response (autoantibodies, etc.), or due to malignancy (for example, interference by paraproteins with phosphate testing and indirect ion selective electrode methods) may show some negative effects that will cause various attempts and some misjudgements.[28]

These performance characteristics have been obtained using an autoanalyzer. Results may vary slightly when using different equipment or manual procedures.

WARNINGS AND PRECAUTIONS

IVD: For in Vitro Diagnostic use only.
Do not use expired reagents.
Reagents with two different lot numbers should not be interchanged.
For professional use.
Follow Good Laboratory Practice (GLP) guidelines.

CAUTION: Human source samples are processed with this product. All human source samples must be treated as potentially infectious materials and must be handled in accordance with OSHA (Occupational Safety and Health Administration) standards.

Danger

EUH032 :Releases a very toxic gas if contacts with acid.
H317 :May cause allergic skin reaction.

Precaution

P280	:Use protective gloves / clothes / glasses / mask.
P264	:Wash your hands properly after using.
P272	:Contaminated work clothes should not be allowed to be used outside of the workplace.

Intervention

P302+P352	:Wash with plenty of water and soap if it contacts with skin.
P333+P313	:Seek medical help if it irritates your skin or develops rash.
P362+P364	:Remove contaminated clothes and wash properly before using.

Disposal

P501	:Dispose the vials and contents according to the local regulations.

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SYMBOLS

IVD	In Vitro Diagnostic Medical Device
LOT	Lot Number
R1	Reagent 1
R2	Reagent 2
GTIN	Global Trade Item Number
REF	Reference Number
GLP	Good Laboratory Practice
FOR USE WITH	Identifies Products to Be Used Together
PRODUCT OF TURKEY	Product of Turkey
	Manufacturer
	Expiration Date
	Temperature Limits
	Consult Instructions for Use
	Caution
	Number of Tests