Gcell

Creatinine Assay Kit (Cre)

Method: Creatininase Enzymatic

Cat . No.	Size	Instrument
GB9300S	R1: 2×90 ml R2: 1×60 ml	For Hitachi 717 &ShimadzuCL7200/8000
GS9301S	R1: 3×60 ml R2: 1×60 ml	For Hitachi917 &OlympusAU640/400/600
GH9301S	R1: 2×48 ml R2: 2×16 ml	For Hitachi902
GX9301S	R1: 2×60 ml R2: 2×20 ml	For SYNCHRON CX4-5-7-9/ LX20/DXC600-800

INTENDED USE

For the *in vitro* quantitative determination of Creatinine in serum, plasma or urine. This product is suitable for Manual use. This product is suitable for use on Hitachi 704/717/737/902/ 904/911/912/917, Synchron CX4/5/7/9/LX20, Olympus400/640/2700 and all automatic analyzer.

CLINICAL SIGNIFICANCE[1,2]

Creatinine is a break-down product of creatine phosphate in muscle, and is usually produced at a fairly constant rate by the body (depending on muscle mass).

High creatinine blood levels can mean serious kidney damage or disease is present. Other conditions that can cause high blood creatinine levels include blockage of the urinary tract (such as by kidney stone), heart failure, dehydration, excessive blood loss that causes shock, gout, or muscle conditions (such as rhabdomyolysis, gigantism, acromegaly, myasthenia gravis, muscular dystrophy, and polymyositis).

Urine creatinine is increased in hypothyrosis, wasting disease, dermatomyositis, tetanus or typhus, but decreased in amyotrophy or leukemia.

ASSAY PRINCIPLE [3]

The enzymatic method involves a series of coupled enzymatic reactions.

First step: the endogenous creatine is eliminated by Creatinase, sarcosine oxidase.

Second step: creatinine in the specimen is converted to creatine by creatininase, and then the product creatine is hydrolyzed to sarcosine by creatinase, followed by the oxidation of sarcosine by sarcosine oxidase (SOD) producing hydrogen peroxide (H_2O_2) which is quantified by a Trinder reaction.

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

$$\text{Creatine} + H_2O \xrightarrow{\text{Creatinase}} \text{Sarcosine} + \text{Urea}$$

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

$$2H_2O_2 + \text{EMSE} + \text{4-AAP} + H_3^+O \xrightarrow{\text{POD}} \text{pigment} + 5H_2O$$

EMSE:
N-ethyl-N-(3-methylphenyl)-N'-succinylethylenediamine

SPECIMEN COLLECTION

Serum, plasma or urine.

Urine: diluted 1 + 9 with redistilled water.
Serum / plasma creatinine is stable for 7 days at 20 - 25°C, 7 days at 4 - 8°C and 3 months at - 20°C.
Urine creatinine is stable for 2 days at 20 - 25°C, 6 days at 4 - 8°C and 6 months at - 20°C.

REAGENT COMPOSITION

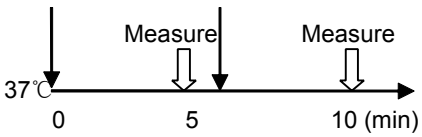
Contents	Concentration
Reagent 1	
creatinase	60 U/ml
sarcosine oxidase	15 U/ml
EMSE	1.4 mmol/L
Buffer（pH7.7）	50 mmol/L
Reagent 2	
Peroxidase	30 U/ml
creatininase	310 U/ml
4-aminoantipyrine	2.5 mmol/L
Buffer（pH7.7）	50 mmol/L

STABILITY AND PREPARATION OF SOLUTIONS

All reagents are ready to use.
Stable up to the expiry date when stored at 2-8℃.
Once opened contents are stable for 1 month at 2-8℃.

ASSAY PROCEDURE

Test Procedure for Analyzers (HITACHI 7170/917)
Assay Mode: 2 Point END, 16-34
Wave length (main/sub): 546 nm/700 nm
Sample:4 µl
R1: 210 µl R2:70µl



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1. Mix 4 µl sample with 210 µl R1 and incubate at 37℃ for 5 minutes, then read initial absorbance A_1 at 546nm.
2. Add 70 µl R2 into cuvette, mix and incubate for 5 minutes at 37℃, Read final absorbance A_2 .
3. Calculate the absorbance change $\Delta A=A_2-A_1$.

CALCULATION

$$\text{Concentration} = \frac{\Delta A_{sample} - \Delta A_{blank}}{\Delta A_{calibrator} - \Delta A_{blank}} \times \text{Calibrator value}$$

CALIBRATION

Calibrator (value is lot specific) provided with the kit is recommended for calibration. The calibrator is traceable to NIST SRM 967a and should be stored at 2–8°C.

QUALITY CONTROL

For accuracy and reproducibility control: Randox Controls are recommended.
Two levels of controls should be assayed at least once a day. Values should fall within a specific range. If these values fall outside the range and repetition excludes error, the following steps should be taken:

1. Check instrument settings and light source.
2. Check reaction temperature.
3. Check expiration date of kit and contents.

Total Precision					
Serum Testing	HN1530	HE1532	Serum 1	Serum 2	Serum 3
NO.of Data Points	80	80	80	80	80
Mean (µmol/L)	128.3	375.8	79.8	184.1	379.5
SD (µmol/L)	1.34	3.53	1.11	1.92	4.17
Cv%	1.04	0.94	1.39	1.04	1.1

CONVERSION FACTORS

mg/dl ×88.4= µmol/L

NORMAL RANGE

Serum/plasma (adults)
Men:59–104µmol/L(0.67–1.17mg/dl)
Women:45–84µmol/L(0.51–0.95mg/dl)

1st morning urine (adults)
Men: 3540–24600µmol/L(40–278mg/dl)
Women: 2550–20000µmol/L(29–226 mg/dl)

24-hour urine (adults)
Men: 8.6 – 19.4mmol/24h (980 – 2200 mg/24h)
Women: 6.3 – 13.4mmol/24h (720 – 1510mg/24h)

Creatinine clearance
66–143ml/min

It is recommended that each laboratory should assign its own normal range as this is dependent upon geographical location.

ASSAY RANGE

The method is linear between creatinine concentrations of approximately 0.113 - 135 mg/dl (10 - 12000 umol/L). Sample above the top concentration should be diluted with 0.9% NaCl and reassay. Multiply the result by dilution factor.

SPECIFIC PERFORMANCE CHARACTERISTICS

SPECIFICITY

A Reagent blank may be performed by replacing sample or standard with double deionized water. The following analytes were tested up to the levels indicated and found not to interfere:

Hemoglobin	200 mg/dl
Intralipid	1000 mg/dl
Ascorbic Acid	50 mg/dl
Direct bilirubin	40 mg/dl

Sensitivity

The limit of detection is 5 µ mol/L (0.06 mg/dl).

PRECISION

The precision was evaluated according to Clinical Laboratory Standards Institute EP05-A2 guideline. In the study, two Randox Controls and three serum specimens were tested twice daily, in duplicates over 20 days.

Correlation

Serum
A comparison of the creatinine determination using the Gcell Creatinine assay(y) with a commercially creatinine PAP method (x) gave the following correlation (µmol/L):
Linear regression: y = 1.005x - 0.760
r =0.999
Number of samples measured : 137
The sample concentrations were between 38.8 and 1844µmol/L.

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Urine
A comparison of the creatinine determination using the Gcell Creatinine assay(y) with a commercially creatinine PAP method (x) gave the following correlation (µmol/L):
Linear regression: y = 0.998x -5.195
r =0.999
Number of samples measured: 40
The sample concentrations were between 2983 and 25722 µmol/L.

SAFETY PRECAUTIONS AND WARNINGS

1. For *in vitro* diagnostic use only. Do not pipette by mouth. Exercise the normal precautions required for handling laboratory reagents.
2. Reagent 2 contains Sodium Azide. Avoid ingestion or contact with skin or mucous membranes. In case of skin contact, flush affected area with copious amounts of water.In case of contact with eyes or if ingested, seek immediate medical attention.
3. Sodium Azide reacts with lead and copper plumbing, to form potentially explosive azides. When disposing of such reagents flush with large volumes of water to prevent azide build up. Exposed metal surfaces should be cleaned with 10% sodium hydroxide.
4. All specimens used in this test should be considered potentially infectious. Universal Precautions, as they apply at your facility, should be used for handling and disposing of materials during and after testing.

REFERENCES

1. Chernecky CC, Berger BJ, eds. (2004). Laboratory Tests and Diagnostic Procedures, 4th ed. Philadelphia: Saunders.
2. Fossati P, Prencipe L, Berti G. Enzymatic creatinine assay: a new colorimetric method based on hydrogen peroxide measurement. Clin Chem 1983, 29:1494-1496.
3. Mazzachi BC, Peake M, Erhardt V. Reference range and method comparison studies for enzymatic and Jaffé Creatinine assays in plasma and serum and early morning urine. Clin Lab 2000, 46:53-55.
4. Junge W, Wilke B, Halabi A, Klein G. Determination of reference intervals for serum creatinine, creatinine excretion and creatinine clearance with an enzymatic and amodified Jaffé method. Clin Chim Acta 2004, 344:137-148.

INDEX OF SYMBOLS

	Manufacture
REF	Catalogue Number
LOT	Lot number
	Date of manufacture
	Use by (Expiration date)
IVD	For In-Vitro Diagnostic use only
2℃ 8℃	Stored at 2-8℃
	Attention: See instruction for use
EC REP	Authorized Representative in the European Company

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INSTRUMENT SETTINGS FOR HITACHI 917

Hitachi 7170 Parameter Application — **Gcell**

CRE
Cat. No: GS9301S-GB9300S

Analysis

Test / Type	CRE						Ser/Pl		
Assay/Time/Point	2Point End		A	10	A	16	34	0	0
Wave (Sub/Main)	700	A	546	A					
S.Vol (Normal)	4	0.0	0						
S.Vol (Decrease)	2	0.0	0						
S.Vol (Increase)	10	0.0	0						
Diluent	Water		0						
Reagent (R1) T1	200	0	*	0					
Reagent (R2) T2	0	0	00000	0					
Reagent (R3) T3	70	0	*	0					
Reagent (R4) T4	0	0	00000	0					
Abs. Limit	32000	Increase		A					
Prozone Limit	0	34	Lower	A					
Cell Detergent	Detergent 1	A							

Range

Application Code	*	Unit	umol/L	A		
Report Name	CRE					
Data Mode	On Board	A				
Control Interval	0					
Instrument Factor (Y=aX+b) a=	1.0	b=	0			
Technical Limit	0	1320				
Expected Value						
				Qualitative		
(Male)	0	Y	A		Cancel	A
	100	Y	A		(1)	0
			22.1	106	(2)	0
(Female)	0	Y	A		(3)	0
	100	Y	A		(4)	0
			17.7	106	(5)	0

Calibration

Calibration type	Linear	A		A
Point	2	Span Point	2	
Weight	0			
Auto calibration				
Time Out		Change Over		
Blank	0	Blank	A	
Span	0	Blank	A	
2Point	0			
Full	0			
SD Limit	0.1			
Duplicate limit	1000			
Sensitivity limit	0			
S1 Abs limit	-32000	32000		

STD Conc

<Standard>	(1)	(2)	(3)	(4)	(5)	(6)
Concentration	0	*	0	0	0	0
Position	Water	*	0	0	0	0
Volume	4	4	0	0	0	0
<Pre-Diluent>						
Volume	0	0	0	0	0	0
Diluent	0	0	0	0	0	0
Cal. Code	0	0	0	0	0	0

Attention: * entered by operator
K-factor =

INSTRUMENT SETTINGS FOR Olympus400/640/2700

Olympus AU640/400/2700 Instrument Settings — **Gcell**

CRE
Cat. No: GS9301S/GB9300S

Specific Test Parameters

Test Name:	Cr			Type:	Serum	Operation:	Yes
Sample: Volume	4.5	Dilution	0	Pre-Dilution Rate:			
Reagents: R1 Volume	240	Dilution	0	Min OD		Max OD	
R2 Volume	80	Dilution	0	L	-2.0000	H	2.5000
				Reagent OD Limit:			
Wavelength: Pri.	540	Sec.	800	First L	-2.0000	First H	2.5000
Method:	END			Last L	-2.0000	Last H	2.5000
Reaction Slope:	+			Dynamic Range:			
Measuring Point 1: First	10	Last	27	L	0	H	4862
Measuring Point 2: First		Last		Correlation Factor:			
Linearity:	%			A	1.0	B	0.0
No-Lag-Time:	NO			Onboard Stability Period:			

Calibration Specific

Test No.:		Name:	Cr	Type:	SER
Cal. Type:	AB			Counts:	2
Formula:	Y = AX + B			Process:	CONC
Calibration Selection:					

	Cal. No.	OD	Conc.	Factor/OD-L	Factor/OD-H
Point 1	*		*	-9999999	9999999
Point 2					
Point 3					
Point 4					
Point 5					
Point 6					
Point 7					
1-Point Cal..Point:					
MB Type Factor:					
Cali. Stability Period:					

Attention: * Entered By Operator

INSTRUMENT SETTINGS FOR HITACHI 902

Hitachi 7020 Instrument Settings — **Gcell**

CRE

Cat. No: GB9300S/GS9301S/GH9301S

No.	<Chemistry>	
1	Test Name	CRE
2	Assay Code (Mthd)	2 Point End
3	Assay Code (2. Test)	0
4	Reaction Time	10
5	Assay Point 1	17
6	Assay Point 2	35
7	Assay Point 3	0
8	Assay Point 4	0
9	Wave Leng. (SUB)	700
10	Wave Leng. (MAIN)	546
11	Sample Volume	4
12	**R1** VOLUME	200
13	R1 Pos.	*
14	R1 Bottle Size	Large
15	**R2** VOLUME	0
16	R2 Pos.	0
17	R2 Bottle Size	Small
18	**R3** VOLUME	70
19	R3 Pos.	*
20	R3 Bottle Size	Small
21	Calib. Type (Type)	Linear
22	Calib. Type (Wght)	0
23	Calib. Conc. 1	0
24	Calib. Pos. 1	99
25	Calib. Conc. 2	*
26	Calib. Pos. 2	*
27	Calib. Conc. 3	0
28	Calib. Pos. 3	0
29	Calib. Conc. 4	0
30	Calib. Pos. 4	0
31	Calib. Conc. 5	0
32	Calib. Pos. 5	0
33	Calib. Conc. 6	0
34	Calib. Pos. 6	0
35	S 1 ABS.	0
36	K Factor	10000
37	K 2 Factor	10000
38	K 3 Factor	10000
39	K 4 Factor	10000
40	K 5 Factor	10000
41	A Factor	0
42	B Factor	0
43	C Factor	0
44	SD Limit	999
45	Duplicate Limit	1000
46	Sens. Limit	0
47	S 1 ABS Limit (L)	-32000
48	S 1 ABS Limit (H)	32000
49	ABS Limit	0
50	ABS Limit (D/I)	Increase
51	Prz. Limit	0
52	Prz. Limit (U/D)	Lower
53	Prz. (End Point)	35
54	Expect. Value (L)	22
55	Expect. Value (H)	88
56	Instr. Fact. (a)	1
57	Instr. Fact. (b)	0
58	Key Setting	*

* Data entry by the user

INSTRUMENT SETTINGS FOR CX4/5/7/9

Synchron CX-4/5/7/9 User-defined Chemistries — **Gcell**

CRE

Cat. No: GB9300S/GS9301S/GX9301S

USER ID:
Chemistry Name: CRE
Test Name: CRE

Calculate Factor:

Reaction Type: Endpoint 2
Reaction Direction: POSITIVE
Units: Umol/L

Math Model: Linear
Cal Time Limit: 336 Hrs
No. Of Calibrators: 2

Decimal Precision: X

Primary Wavelength: 560 nm
Secondary Wavelength: 700 nm

Sample Volume: 5 µl
Primary Inject Rgt:
A: 225 µl
None: µl
Secondary Inject Rgt:
B: 75 µl
Add Time: 624 sec

CALIBRATORS
#1: 0
#2: *
#3:
#4:
#5:

MULTIPOINT SPAN
1 - 2 0.000
2 - 3 0.000
3 - 4 0.000
4 - 5 0.000
5 - 1 0.000

RAGENT BLANK
Start Read: 580 sec
End Read: 600 sec
Low ABS Limit: -1.500
High ABS Limit: 1.500

REACTION
Start Read: 600 sec
End Read: 616 sec
Low ABS Limit: -1.500
High ABS Limit: 1.500

USABLE RANGE
Lower Limit: 0
Upper Limit: 1320

SUBSTRATE DEPLETION
Initial Rate: 99. 999
Delta ABS: 1. 5

RECOVERY/SENSITIVITY
Std Dev (conc): *
CV (%): *
Std Dev (mA): *
Threshold: *

Attention: * Entered By Operator

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