Gcell

5'-Nucleotidase Assay Kit (5'-NT)

Method: Colorimetric (Kinetic)

Cat .No.	Size	Instrument
GB8010G	R1: 2 × 20 ml R2: 1 × 20 ml	For Hitachi 717 & ShimadzuCL7200/8000
GS8011G	R1: 2 × 20 ml R2: 1 × 20 ml	For Hitachi917 & OlympusAU640/400/600
GX8011G	R1: 2 × 20 ml R2: 1 × 20 ml	For SYNCHRON CX4-5-7-9/LX20

INTENDED USE

For the *in vitro* quantitative determination of 5'-nucleotidase activity in human serum or plasma.

CLINICAL SIGNIFICANCE

5'-NT is an enzyme catalyzing the hydrolysis of nucleoside-5'-monophosphates to nucleosides and inorganic phosphate. The enzyme is widely distributed in human and animal tissues. The activity present in sera is released from the membrane of liver cells by bile salts and has been used as a marker for liver disease[1]. Increased enzyme levels in sera are associated with certain forms of liver disease, such as intra or extra-hepatic obstruction and particularly in cases of hepatic carcinoma as well as in mastectomy patients with recurrent metastases. The diagnostic value of 5'-NT has been shown to be superior to other liver enzymes, especially in cases of liver metastasis .

ASSAY PRINCIPLE

The 5'-NT assay is based on the enzymatic hydrolysis of 5'-monophosphate (5'-IMP) to form inosine which is converted to hypoxanthine by purine nucleoside phosphorylase (PNP). Hypoxanthine is then converted to uric acid and hydrogen peroxide (H_2O_2) by xanthine oxidase (XOD). H_2O_2 is further reacted with N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline (EHSPT) and 4-aminoantipyrine (4-AA) in the presence of peroxidase (POD) to generate quinone dye which is monitored in a kinetic manner. The entire enzymatic reaction scheme is shown below.

$$IMP + H_2O \xrightarrow{5'\text{-NT}} Inosine + Pi$$

$$Inosine + Pi \xrightarrow{PNP} Hypoxanthine + Ribose\ 1\text{-}phosphate$$

$$Hypoxanthine + 2H_2O + 2O_2 \xrightarrow{XOD} Uric\ acid + 2H_2O_2$$

$$2H_2O_2 + 4\text{-}AA + EHSPT \xrightarrow{POD} 4H_2O + Quinone\ dye$$

(λ_{max}=556 nm)

SAMPLE COLLECTION AND PREPARATION

Serum or plasma samples.
Use fresh and non-hemolyzed serum or EDTA treated plasma samples. Serum or plasma samples are stable for a week at 4°C.

REAGENT COMPOSITION

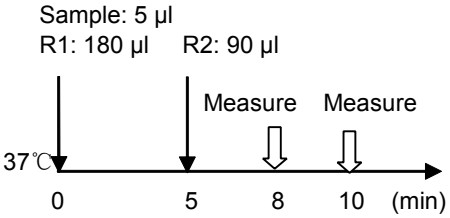
Contents	Concentration of Solutions
Reagent 1 (R1)	
Goods buffer (PH7.6)	100 mmol/L
4-AA	2 mmol/L
PNP	0.1 U/L
XOD	0.2 U/L
POD	0.6 U/L
Stabilizer	
Reagent 2 (R2)	
Goods buffer (PH7.6)	100 mmol/L
IMP	10 mmol/L
EHSPT	2 mmol/L

STABILITY AND PREPARATION OF REAGENTS

All reagents are ready to use.
Stable up to the expiry date when stored at 2-8℃.
The assay kit reagents are stable for 1 month after opening and kept at 2-8℃.

ASSAY PROCEDURE

Test Procedure for Analyzers (HITACHI 7170/917)
Assay Mode: 2 Point Rate 28-34
Wave Length (main/sub): 546 nm/800 nm



1. Mix 5 µl sample with 180 µl R1 and incubate at 37℃ for 5 minutes.
2. Add 90 µl R2 into cuvette, mix and incubate for 3 minutes at 37℃.
3. Read initial absorbance and start timer simultaneously, read again after 1 and 2 minutes.
4. Calculate absorbance change per minute (ΔA/min)

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CALCULATION (ε=16.18)

$$5'\text{-NT (U/L)} = \frac{\Delta A/\min \times V_t}{\varepsilon \times V_s \times L} = \Delta A/\min \times 3400$$

QUALITY CONTROL

For quality control, use 5'-NT control as daily quality control sera and can be purchased separately. 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.

REFERENCE VALUE

Serum or plasma: 0-10 U/L

It is recommended that each laboratory establish its own reference range to reflect the age, sex, diet and geographical location of the population.

LINEARITY

The method is linear up to 300 U/L. Samples above this concentration should be diluted 1+1 with 0.9% NaCl and reassayed. Multiply the result by 2.

SPECIFIC PERFORMANCE CHARACTERISTICS

INTERFERENCE

Hemoglobin≤ 500 mg/dl, Intralipid ≤1000 mg/dl ALP≤1250 U/l, without interference.

Bilirubin≤40 mg/dl, Ascorbic Acid≤50 mg/dl, interference.

PRECISION

The CV of the test should be ≤10%.

Intra assay precision		
N=20	Level1	Level 2
Mean	7.5	78.8
SD	0.11	0.95
CV	1.5%	1.2%
Inter assay precision		
N=5	Level1	Level 2
Mean	7.83	79.3
SD	0.16	1.35
CV	2.0%	1.7%

SENSITIVITY

The minimum detectable level that can be distinguished from zero has been determined as 1.81 U/L.

CORRELATION

This method (Y) was compared with another commercially available method (X) and the following linear regression equation obtained: Y=1.0426X+1.3074, R^2=0.9912; 64 patient samples were analyzed .

SAFETY PRECAUTIONS AND WARNINGS

1. For *in vitro* diagnostic use only. Do not pipette by mouth. Exercise the normal precautions required for handing laboratory reagents.
2. Reagents contain 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. Eroglu A. Activities of adenosine deaminase and 5' - nucleotidase in cancerous and noncancerous human cdorectal tissues[J]. Med Oneol 2000; 17(4) :319 - 24.1
2. Alain Bertrand and Jean Buret, A one-step determination of 5'-nucleotidase using a centrifugal analyzer. Clinica Chimica Acta,119(1982)275-284.
3. By Z. Ahmed and J.L.Reis, The Activation and Inhibition of 5-Nucleotidase. Clin Chem. 1998，69（11），102－106.

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INDEX OF SYMBOLS

	Manufacture
REF	Catalogue Number
LOT	Lot number
	Date of manufacture
	Use by (Expiration date)
IVD	For In-Vitro Diagnostic use only
	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**

5'-NT
Cat. No: GB8010G/GS8011G/GX8011G

Analysis

Test / Type	5NT						Ser/Pl		
Assay/Time/Point	Rate A			A	10	A	28	34	0	0
Wave (Sub/Main)	800	A	546	A						
S.Vol (Normal)	5	0.0	0							
S.Vol (Decrease)	2	0.0	0							
S.Vol (Increase)	10	0.0	0							
Diluent	Water		0							
Reagent (R1) T1	180	0	*	0						
Reagent (R2) T2	0	0	00000	0						
Reagent (R3) T3	90	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	U/L	A		
Report Name	5NT					
Data Mode	On Board	A				
Control Interval	0					
Instrument Factor (Y=aX+b)	a=	1.0	b=	0		
Technical Limit	0	300				
Expected Value						
			Qualitative			
(Male)	0	Y	A		Cancel	A
	100	Y	A		(1)	0
			0	10	(2)	0
(Female)	0	Y	A		(3)	0
	100	Y	A		(4)	0
			*	*	(5)	0

Calibration

Calibration type	Linear	A		A
Point	1	Span Point	0	
Weight	0			
Auto calibration				
Time Out		Change Over		
Blank	0	Blank	A	
Span	0	Blank	A	
2Point	0			
Full	0			
SD Limit	999			
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	0	0
Position	Water	0	0	0	0	0
Volume	5	0	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 = 34000

INSTRUMENT SETTINGS FOR Olympus400/640/2700

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

5'-NT
Cat. No:GB8010G/GS8011G/GX8011G

Specific Test Parameters

Test Name:	5'NT			Type:	Serum	Operation:	Yes
Sample: Volume	5	Dilution	0	Pre-Dilution Rate:			
Reagents: R1 Volume	180	Dilution	0	Min OD		Max OD	
R2 Volume	90	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:	RATE			Last L	-2.0000	Last H	2.5000
Reaction Slope:	+			Dynamic Range:			
Measuring Point 1: First	21	Last	27	L	0	H	300
Measuring Point 2: First		Last		Correlation Factor:			
Linearity:	30%			A	1.0	B	0.0
No-Lag-Time:	YES			Onboard Stability Period:			

Calibration Specific

Test No.:		Name:	5'NT	Type:	SER
Cal. Type:	MB			Counts:	2
Formula:		Y = AX + B		Process:	CONC
Calibration Selection:					

	Cal. No.	OD	Conc.	Factor/OD-L	Factor/OD-H
Point 1					
Point 2					
Point 3					
Point 4					
Point 5					
Point 6					
Point 7					
1-Point Cal.Point:					
MB Type Factor:		3400			
Cali. Stability Period:					

Attention: * Entered By Operator

INSTRUMENT SETTINGS FOR HITACHI 902

Hitachi 7020 Instrument Settings — **Gcell**

5'-NT

Cat. No: GB8010G/GS8011G/GX8011G

No.	<Chemistry>	
1	Test Name	5'-NT
2	Assay Code (Mthd)	Rate A
3	Assay Code (2. Test)	0
4	Reaction Time	10
5	Assay Point 1	27
6	Assay Point 2	34
7	Assay Point 3	0
8	Assay Point 4	0
9	Wave Leng. (SUB)	800
10	Wave Leng. (MAIN)	546
11	Sample Volume	5
12	**R1** VOLUME	180
13	R1 Pos.	*
14	R1 Bottle Size	Large
15	**R2** VOLUME	0
16	R2 Pos.	0
17	R2 Bottle Size	Small
18	**R3** VOLUME	90
19	R3 Pos.	*
20	R3 Bottle Size	Small
21	Calib. Type (Type)	K Factor
22	Calib. Type (Wght)	0
23	Calib. Conc. 1	0
24	Calib. Pos. 1	99
25	Calib. Conc. 2	0
26	Calib. Pos. 2	0
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	3400
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	32000
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)	0
55	Expect. Value (H)	10
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**

5'-NT

Cat. No: GS8011G/GB8010G/GX8010G

USER ID:
Chemistry Name: 5'-NT
Test Name: 5'-NT

Calculate Factor: 6800

Reaction Type: Rate 1
Reaction Direction: Positive
Units: U/L

Math Model: Linear
Cal Time Limit: 0 Hrs
No. Of Calibrators: 0

Decimal Precision: X.X

Primary Wavelength: 560 nm

Secondary Wavelength: 700 nm

Sample Volume: 4 µl
Primary Inject Rgt:
A: 144 µl
B: 72 µl
Secondary Inject Rgt:
None: 0 µl
Add Time: sec

CALIBRATORS
#1:
#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: 260 sec
End Read: 300 sec
Low ABS Limit: -1.500
High ABS Limit: 1.500

REACTION
Start Read: 180 sec
End Read: 300 sec
Low ABS Limit: -1.500
High ABS Limit: 1.500

USABLE RANGE
Lower Limit: 0
Upper Limit: 300

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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