



skyla

Diagnosis-II Panel



PN : 900-320

For Veterinary In Vitro Diagnostic Use Only

Rev : D

1. Intended Use

The skyla Diagnosis-II Panel used with s skyla Analyzer, is intended to be used for the quantitative determination of Albumin (ALB), Alkaline Phosphatase (ALP), Alanine Aminotransferase (ALT), Amylase (AMY), Blood Urea Nitrogen (BUN), Creatinine (CREA), Glucose (GLU), Total bilirubin (TBIL), Total cholesterol (CHOL), Total Protein (TP), Calcium (Ca), Phosphorus (PHOS), Lipase (LIPA), and Gamma-Glutamyl Transpeptidase (GGT) in animal whole blood, plasma, or serum. The calculated values of Globulin (GLOB), UREA, Albumin/Globulin Ratio (A/G Ratio), Blood Urea Nitrogen/Creatinine Ratio (B/C Ratio) and Corrected Calcium(C-Ca) can then be obtained.

2. Principles

The skyla Diagnosis-II Panel contains a total of 14 types of dried reagents located in the respective detection wells of the reagent disc. The user only needs to inject the blood specimens into the sample port of the disc, and then places the disc into the analyzer. The test will be done automatically within 15 minutes. Three additional calculated values are also obtained after the test. For the detail description of disc, please refer to “skyla Analyzer Operator’s Manual”.

Clinical Significance:

Albumin (ALB): ALB is one of the indicators for kidney function, liver function and dehydration.

Alkaline phosphatase (ALP): ALP is one of the indicators for liver and biliary related diseases.

Alanine Aminotransferase (ALT): ALT is used to detect pet viral hepatitis, cirrhosis, and the degree of liver injury and related diseases.

Amylase (AMY): AMY is one of the indicators for acute pancreatitis and kidney diseases.

Blood Urea Nitrogen (BUN): BUN is one of the important markers for diagnosis and prognosis tracking of kidney diseases.

Creatinine (CREA): CREA is a marker to examine renal functions.

Glucose (GLU): GLU can be used for the diagnosis of diabetes and diseases related to the

carbohydrate metabolism.

Total bilirubin (TBIL): TBIL can be used for the diagnosis of obstructive liver diseases and hepatobiliary diseases.

Total Cholesterol (CHOL): CHOL test can be used to assess the metabolic state of lipids.

Total Protein (TP): TP is an indicator for function of liver synthesis and the degree of protein-losing caused by kidney diseases.

Calcium (Ca): Ca can be used to detect parathyroid-related bone diseases, chronic kidney diseases and tetany of vitamin D deficiency.

Phosphate (PHOS): PHOS is an indicator for kidney diseases, hypothyroidism, and malnutrition.

Lipase (LIPA): LIPA is a reliable marker in diagnosis of pancreatic disease.

Gamma-Glutamyl Transpeptidase (GGT): GGT can be used for the diagnosis of liver disease, primary and secondary liver tumors.

Globulin (GLOB): GLOB is calculated from TP and ALB and it is used to assess liver function.

UREA : UREA is synthesized in the liver and secreted by the kidneys. Urea is the end product of protein nitrogen metabolism and is the primary vehicle for removing toxic ammonia from the body. The analysis of urea is an important clinical test for renal disease and dysfunction.

Albumin/Globulin Ratio (A/G Ratio): The A/G Ratio is the ALB and GLOB ratio. It is used to assess liver function.

Blood Urea Nitrogen/ Creatinine Ratio (B/C Ratio): The B/C Ratio may indicate the degree of kidney injury and azotemia.

Corrected Calcium (C-Ca): C-Ca is calculated from Ca and ALB and it is used to assess Hypocalcaemia

Method:

ALB

ALB is determined through the endpoint chemical reaction method. When ALB binding to Bromocresol Green (BCG), it forms a yellow-green complex. The absorbance at a wavelength of 600 nm can be measured. The amount of ALB in the sample is proportional to the bound ALB.

ALP

ALP activity is enzymatically determined. *p*-Nitrophenyl Phosphate that is hydrolyzed by ALP into a yellow colored product *p*-Nitrophenol which has an absorbance at a wavelength of 405 nm. The rate of the reaction is directly proportional to the enzyme activity.

ALT

ALT activity is enzymatically determined. ALT catalyses the alanine with α -Ketoglutarate, and converts them into Glutamate and Pyruvate. In the presence of NADH, Lactate Dehydrogenase converts Pyruvate into Lactate. In the course of the reaction NADH is oxidized to NAD. The

decrease of NADH absorbance is measured at a wavelength of 340 nm and is proportional to ALT activity

AMY

Amylase activity is enzymatically determined. The substrate α -(2-Chloro-4-Nitrophenyl)- β -1,4-Galactopyranosylmaltoside (Gal-G2- α -CNP) reacts directly with α -Amylase and releases 2-Chloro-4-Nitrophenol (CNP) from the substrate. The resulting absorbance is measured at a wavelength of 405 nm and is directly related to the α -Amylase activity in the sample.

BUN

BUN is enzymatically determined. Urea undergoes an Urease catalyzed hydrolysis, thus producing Ammonia and Carbon Dioxide. In a Glutamate Dehydrogenase (GLDH) catalyzed reaction, Ammonia reacts with 2-Oxoglutarate yielding L-Glutamate. In the process of this reaction, β -Nicotinamide Adenine Dinucleotide (NADH) is oxidized to β -Nicotinamide Adenine Dinucleotide (NAD⁺) which in turn undergoes a color reaction. The rate of change of absorbance at a wavelength of 340 nm is measured and proportional to the BUN concentration.

CREA

CREA is determined through the endpoint enzymatic reaction approach. Creatinine Amidohydrolase hydrolyzes CREA to Creatine. Then Creatine is converted into Sarcosine through catalysis of Creatine Amidinohydrolase. Furthermore, Sarcosine Oxidase oxidizes Sarcosine, yielding Glycine, Formaldehyde and Peroxide (H₂O₂) in the process. The enzyme Peroxidase processes Hydrogen Peroxide, 2,4,6-trihydroxy-Benzoic Acid (TBHBA) and 4-Aminoantipyrine (4-AAP), forming a Quinoneimine dye as a product. The dye formation is measured at a wavelength of 546 nm and is proportional to the amount of CREA in the sample.

GLU

GLU is determined through the endpoint enzymatic reaction approach. The Sucrose is catalyzed by Hexokinase to D-Glucose-6-Phosphate (G-6-P). In the presence of NAD, G-6-PD converts G-6-P into 6-Phosphogluconate and NADH. The absorbance at a wavelength of 340 nm can be measured in the presence of NADH. The absorbance is proportional to the GLU concentration.

TBIL

TBIL is determined by the vanadate oxidation method. In a pH3 buffer system, TBIL undergoes oxidation forming Biliverdin. The absorbance at a wavelength of 450 nm is measured and proportional to the total bilirubin concentration in the sample.

CHOL

CHOL is determined enzymatically by an endpoint reaction. It is hydrolyzed by Cholesterol Esterase (COE) into free Cholesterol and Fatty Acids. Cholesterol and NAD reacts with Cholesterol Dehydrogenase (CDH) to produce Cholest-4-En-3-One and NADH. The absorbance at the wavelength of 340 nm can be measured in the presence of NADH. The absorbance is proportional to the TC concentration.

TP

TP is determined by the Biuret method. The peptide bonds of the protein react with copper ions in an alkaline environment and form a purple compound. The color development is proportional to the original TP concentration and is measured at a wavelength of 546 nm.

Ca

Ca is determined through the endpoint chemical reaction approach. Calcium reacts with Arsenazo III and form a purple-colored complex. The complex formation is measured at a wavelength of 650 nm and is proportional to the amount of Ca in the sample.

PHOS

PHOS is enzymatically determined. By going through a series of enzymatic reactions with Sucrose Phosphorylase, Phosphoglucomutase, and Glucose-6-Phosphate Dehydrogenase, PHOS forms 6-Phosphogluconate and NADH. And NADH is measured at a wavelength of 340 nm and is proportional to the amount of PHOS in the sample.

LIPA

LIPA activity is enzymatically determined. The substrate 1,2-O-Dilauryl-Rac-Glycerol-3-Glutaric acid-(6'-Methylresorufin) Ester(DGGMR) reacts directly with LIPA and releases Methylresorufin from the substrate. The resulting absorbance is measured at a wavelength of 546 nm and is directly related to the LIPA activity in the sample.

GGT

GGT is enzymatically determined. GGT catalyzes the reaction between L- γ -Glutamyl-3-Carboxy-4-Nitroanilide and Gly-Gly, and cause the formation of L- γ -Glutamyl-Glycylglycine and 5-Amino-2-Nitrobenzoate with yellow color. The rate of liberation of 5-Amino-2 Nitrobenzoate is directly related to the GGT activity in the sample and is quantitated by measuring the increase in absorbance at wavelength of 405 nm.

Reaction pathway :

ALB

Albumin + BCG \longrightarrow Albumin-BCG Complex

ALP

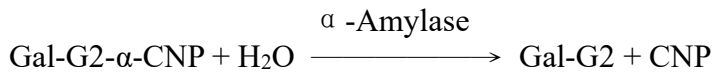
p -Nitrophenyl Phosphate $\xrightarrow{\text{ALP}}$ p -Nitrophenol + Phosphate

ALT

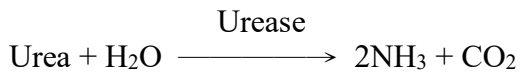
L-Alanine + α -ketoglutarate $\xrightarrow{\text{ALT}}$ Pyruvate + L-Glutamate

Pyruvate + NADH + H⁺ $\xrightarrow{\text{LDH}}$ L-Lactate + NAD⁺ + H₂O

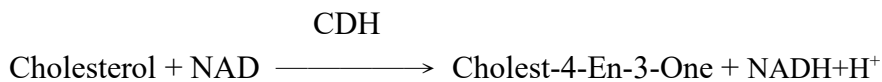
AMY



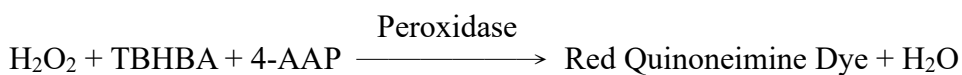
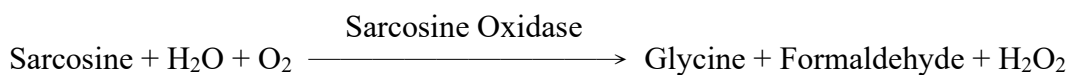
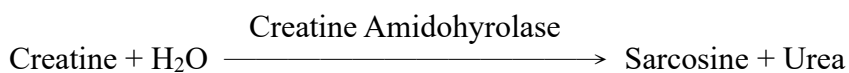
BUN



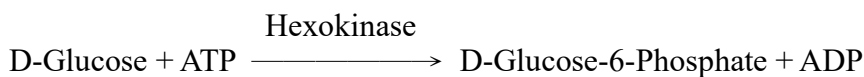
CHOL



CREA



GLU



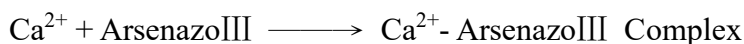
TBIL



TP



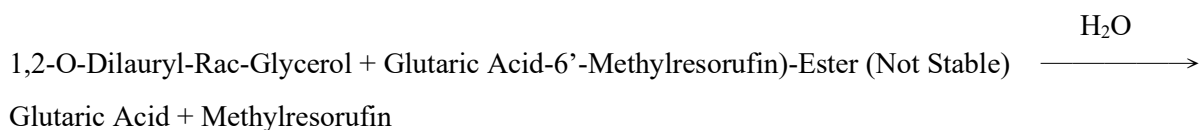
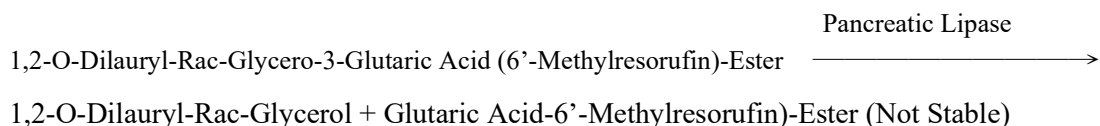
Ca



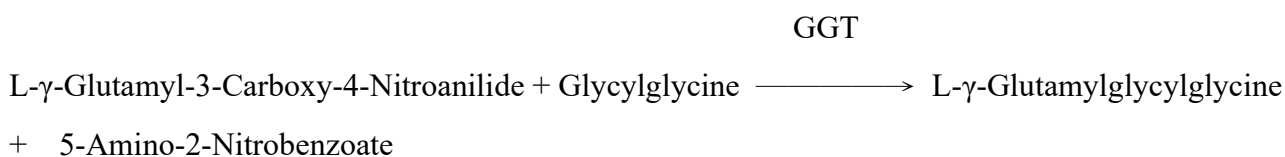
PHOS



LIPA



GGT



3. Reagents

Included:

Each panel contains dried reagent beads, dried internal QC beads and the diluent.

Reagent Composition:

Composition	Quantity/Panel
1,4-Piperazinediethanesulfonic Acid	0.08 mg
4-APP	0.02 mg
4-Nitrophenyl Phosphate Disodium Salt	0.1 mg
Arsenazo III	7 µg

Composition	Quantity/Panel
ATP	0.04 mg
Bromocresol Green	5.4 µg
Cholesterol Dehydrogenase	0.36 U
Cholesterol Esterase	1.44 U
Colipase Porcine Pancreas	0.3 µg
Copper Sulphate	0.1 mg
Creatinase	2.8 U
Creatininase	5.6 U
DGGMR	0.008 mg
G6PDH	0.3 U
Gal-G2-α-CNP	0.04 mg
Glycylglycine	0.38 mg
Hexokinase	0.1 U
Lactate Dehydrogenase	0.3 U
L-Alanine	0.3 mg
L-γ-Glutamyl-3-Carboxy-4-Nitroanilide	0.1 mg
NAD	0.34 mg
NADH	0.06 mg
Peroxidase	0.1 U
Phosphoglucomutase	0.05 U
Sarcosine Oxidase	0.4 U
Sodium Metavanadate	0.01 mg
Sucrose	0.3 mg
Sucrose Phosphorylase	0.01 U
TBHBA	0.2 mg
Urease	0.03 U
α-Ketoglutaric Acid	0.25 mg

Reagent Storage:

- The reagent disc should be stored at 2~8°C.
- The expiry date of the reagent is printed on the outside of the sealed pouch of reagent disc. Do not use if the reagent disc has expired.

4. Specimen Collection and Preparation

Specimen Collection:

- Specimens suitable for skyla Diagnosis-II Panel include lithium heparinized whole blood, lithium heparinized plasma, serum and quality control materials. The sample requirement is 200 µL. (±10 µL tolerance are allowable)
- If applicable, local regulatory or standard operating procedures of your organization should be followed for the collection, preservation and handling of specimens.

Note: Do not use specimens containing other coagulants. That would cause an incorrect test results.

Specimen Preparation:

- Before applying a sample to the reagent disc, gently rotate the sample tube up and down several times, to confirm the sample is homogeneous (evenly mixed). If the sample is whole blood, do not shake the sample container vigorously to avoid occurrence of hemolysis.

Note:

1. Perform testing within 10 minutes after applying the sample to the reagent disc.
2. The use of whole blood specimens with hematocrits (Hct) higher than 60% may affect the test results.

Note: For further information in specimen collection and preparation, please refer to “skyla Analyzer Operator’s Manual”

5. Test Procedures

Material Preparation:

1 piece of the reagent disc of skyla Diagnosis-II Panel

Required materials not included in the panel:

skyla Analyzer

Sample collection container

Micropipette / Tips

Test Conditions:

Test should be carried out in an environment with temperatures of 10°C~32°C. Each test will take about 15 minutes. During the test, chamber in the analyzer keeps the temperature at 37°C for stable assay.

Test Steps:

1. Open the aluminum pouch and remove the reagent disc.
2. Remove the diluent container sealing.
3. Using a micropipette to inject 200 µL of the sample into the reagent disc through the sample port.
4. Press the “start” button on the screen to initiate testing.
5. Place the reagent disc to the analyzer drawer, and press the “ok” button on the screen to

analysis.

For details on the operating steps and instrument setting, please refer to “skyla Analyzer Operator’s Manual”.

Note:

1. To operate the reagent disc or instrument, please wear lab gloves and other protective gear to avoid contamination by specimen.
2. The used reagent disc and tips should be discarded as biomedical waste, and treat according to the local legal requirements.
3. Testing should be performed within 20 minutes after the pouch is opened.
4. Do not place the reagent disc at the environment more than 25°C and longer than 48 hours prior to use.
5. If the reagent disc or its package is damaged or is over the expiry date, do not use it.

6. Calibration

The barcode on every manufactured reagent disc contains all information required for calibration of the test items. The analyzer will automatically read the barcode information during testing.

7. Quality Control

- Please refer to the instruction manual for the preparation and use of quality control materials. For discrepancy results, the confirmatory test was suggested to carry out with the automated laboratory analyzer, or to contact with our technical support.
- External quality control materials can be used for the accuracy monitor of skyla system. The recommended frequency of QC testing is as follows, otherwise please follow local legal requirements or the standard operating procedures of your organization
 - At least every 30 days.
 - Before a new batch of reagents is used for testing.
 - When the analyzer was moved or the operating environment significantly changed.

8. Reference interval

The table below shows the reference interval for each test item. It is recommended that every laboratory or test site should establish its own reference interval from its patient population.

Test Item		Reference Interval		Reference Interval (SI Unit)	
ALB	Canine	2.6-4.6	g/dL	26 - 46	g/L
	Feline	2.5-4.6	g/dL	25 - 46	g/L
ALP	Canine	0-212	U/L	0-212	U/L
	Feline	0-111	U/L	0-111	U/L
ALT	Canine	0-88	U/L	0-88	U/L
	Feline	0-116	U/L	0-116	U/L
AMY	Canine	400 - 1500	U/L	400 - 1500	U/L
	Feline	500 - 1600	U/L	500 - 1600	U/L
BUN	Canine	6.0 - 26.0	mg/dL	2.1 - 9.3	mmol urea/L
	Feline	13.0 - 37.0	mg/dL	4.6 - 13.2	mmol urea/L
CHOL	Canine	110 – 320	mg/dL	2.8 – 8.3	mmol/L
	Feline	54 – 220	mg/dL	1.4 – 5.7	mmol/L
CREA	Canine	0.4-1.6	mg/dL	35-141	μmol/L
	Feline	0.7-2.0	mg/dL	62-177	μmol/L
GLU	Canine	60-110	mg/dL	3.3 - 6.1	mmol/L
	Feline	53 - 150	mg/dL	2.9 - 8.3	mmol/L
TBIL	Canine	0.0-0.9	mg/dL	0.0-15.0	μmol/L
	Feline	0.0-0.9	mg/dL	0.0-15.0	μmol/L
TP	Canine	5.2 - 8.2	g/dL	52 - 82	g/L
	Feline	5.7 - 8.9	g/dL	57 - 89	g/L
Ca	Canine	7.9-12.0	mg/dL	2.2 - 3.0	mmol/L
	Feline	8.0 - 12.0	mg/dL	2.0 - 3.0	mmol/L
PHOS	Canine	2.5 - 6.8	mg/dL	0.8 - 2.2	mmol/L
	Feline	3.1 - 7.5	mg/dL	1.0 - 2.4	mmol/L
LIPA	Canine	25-125	U/L	25-125	U/L
	Feline	25-35	U/L	25-35	U/L
GGT	Canine	<10	U/L	<10	U/L
	Feline	<10	U/L	<10	U/L

9. Limitation

Physiological interferences in blood include hemolysis, icterus, and lipemia. For every test item, 2 Levels serum pool supplemented with known concentrations of the endogenous substances were used for the testing. Significant interference is defined as a >20% shift in the test result. (Note: Highest tested concentration for Hemoglobin: 600 mg/dL; Bilirubin (unconjugated): 62.5 mg/dL, Bilirubin (conjugated): 57.5 mg/dL; Intralipid: 0.55%)

Test Item	Substance concentration with interferences of less than 20%			
	Hemoglobin	Bilirubin (unconjugated)	Bilirubin (conjugated)	Intralipid
ALB	300 mg/dL	62.5 mg/dL	57.5 mg/dL	0.2%
ALP	600 mg/dL	25.9 mg/dL	57.5 mg/dL	0.1%

Test Item	Substance concentration with interferences of less than 20%			
	Hemoglobin	Bilirubin (unconjugated)	Bilirubin (conjugated)	Intralipid
ALT	500 mg/dL	34.5 mg/dL	28.4 mg/dL	0.1%
AMY	600 mg/dL	35.2 mg/dL	19.4 mg/dL	0.2%
BUN	500 mg/dL	42.1 mg/dL	29.3 mg/dL	0.43%
CHOL	300 mg/dL	30.0 mg/dL	30.0 mg/dL	0.2%
CREA	200 mg/dL	25.9 mg/dL	---	0.17%
GLU	600 mg/dL	62.5 mg/dL	57.5 mg/dL	0.3%
TBIL	600 mg/dL	---	---	0.1%
TP	300 mg/dL	62.5 mg/dL	57.5 mg/dL	0.2%
Ca	600 mg/dL	56.3 mg/dL	57.5 mg/dL	0.3%
PHOS	500 mg/dL	42.1 mg/dL	57.5 mg/dL	0.13%
LIPA	200 mg/dL	29.0 mg/dL	20.2 mg/dL	0.2%
GGT	400 mg/dL	36.7 mg/dL	26.3 mg/dL	0.1%

10. Performance Characteristics

Dynamic range:

The dynamic range for each test item showed as below.

Test Item	Dynamic Range		Dynamic Range (SI Unit)	
ALB	1.0-6.0	g/dL	10-60	g/L
ALP	41 - 2000	U/L	41 - 2000	U/L
ALT	20 - 1100	U/L	20 - 1100	U/L
AMY	22 - 3000	U/L	22 - 3000	U/L
BUN	2.0 - 140	mg/dL	0.7 - 50.0	mmol urea/L
CHOL	50-540	mg/dL	1.4 – 14	mmol/L
CREA	0.3-20	mg/dL	27 - 1768	μmol/L
GLU	30 - 550	mg/dL	1.7 - 30.5	mmol/L
TBIL	0.4 - 30.0	mg/dL	6.8 - 513.1	μmol/L
TP	1.5 - 10.0	g/dL	15 - 100	g/L
Ca	4.0 - 15.0	mg/dL	1.0 - 3.8	mmol/L
PHOS	0.4-18	mg/dL	0.03 - 6.45	mmol/L
LIPA	25-300	U/L	25-300	U/L
GGT	10-1500	U/L	10-1500	U/L











Method Comparison:

The SIMENS ADVIA 1800 was used as comparative method in the study. The tests are performed by using the same clinical serum sample for two methods.

The Cobas c 111 was used as comparative method in LIPA study.

Marker	R ²	Slope	Intercept	Sample No.	Sample Range
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Marker		R ²	Slope	Intercept	Sample No.	Sample Range
ALB	Canine	0.9848	0.9999	0.0000	38	2.7-5.9 g/dL
	Feline	0.9676	1.0000	0.0000	38	3.1-6.4 g/dL
ALP	Canine	0.9626	0.9999	-0.0059	32	53-1246 U/L
	Feline	0.9581	0.9998	-0.0010	32	24-263 U/L
ALT	Canine	0.9872	0.9934	-2.4272	32	28-284 U/L
	Feline	0.9951	1.0290	0.2758	32	31-243 U/L
AMY	Canine	0.9955	0.9830	10.544	20	368-2454 U/L
	Feline	0.9925	0.9689	28.25	24	724-2759 U/L
BUN	Canine	0.9967	0.9843	0.6679	41	9.7-128.4 mg/dL
	Feline	0.9923	1.0067	-0.7677	40	17.5-126.9 mg/dL
CHOL	Canine	0.9944	0.9115	2.840	12	98-310 mg/dL
	Feline	0.9899	1.0557	-10.199	15	84-220 mg/dL
CREA	Canine	0.9968	1.0526	-0.0305	38	0.47-16.93 mg/dL
	Feline	0.9928	1.0498	-0.2650	38	1.2-17.65 mg/dL
GLU	Canine	0.9953	1.0001	0.0089	43	78-558 mg/dL
	Feline	0.9957	0.9956	2.1761	44	93-549 mg/dL
TBIL	Canine	0.9966	0.9866	0.2672	23	0.1-31.2 mg/dL
	Feline	0.9954	0.9965	0.0687	25	0.1-31.2 mg/dL
TP	Canine	0.9603	0.9999	0.0000	38	5.2-9.5 g/dL
	Feline	0.9883	0.9999	0.0000	38	6.3-10.3 g/dL
Ca	Canine	0.9945	1.0006	-0.0095	19	7.3-16.4 mg/dL
	Feline	0.9689	0.9814	0.1209	19	7.1-16.4 mg/dL
PHOS	Canine	0.9434	0.9434	0.2678	30	2.7-13.2 mg/dL
	Feline	0.9369	0.9369	0.3763	32	3.3-11.1 mg/dL
LIPA	Canine	0.9932	1.0139	-1.1153	20	27-289 U/L
	Feline	0.9961	0.9977	1.4814	8	26-220 U/L
GGT	Canine	0.9992	1.0014	- 0.5713	28	17-1861 U/L
	Feline	0.9988	1.0027	0.0039	12	27-1647 U/L

Symbol Index			
	Catalogue number		Consult instruction for use
	Batch code		Use by
	Manufacturer		CE mark
	Temperature limitation		Caution
	Do not reuse		Sufficient for

Supplier : SKYLA CORPORATION HSINCHU SCIENCE PARK BRANCH
 Address : 1F, No.8, Dusing Rd., Hsinchu Science Park, East Dist., Hsinchu City, Taiwan
 Customer service/
 Technical support : +886-3-611-8511
 Website : www.skyla.com

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