skyla Single Assay Cartridge

SA15: TRIG+CHOL

For Veterinary In Vitro Diagnostic Use Only

PN：900-215
Rev：E

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1. Intended Use

The skyla TRIG/CHOL single assay cartridge used with skyla Analyzer, is intended to be used for the quantitative determination of Triglyceride (TRIG) and Total Cholesterol (CHOL) in animal plasma or serum.

2. Principles

The skyla TRIG/CHOL single assay cartridge contains a dried reagent. The user only needs to put the cartridges on the single assay carrier, injects the diluted specimens into the sample ports of the cartridges, and then places the carrier into the analyzer. The test will be done automatically within 10 minutes. For the detail description of disc, please refer to “skyla Analyzer Operator’s Manual”.

Clinical Significance:

Triglyceride (TG): TG test can be used to assess the metabolic state of lipids.

Total Cholesterol (CHOL): CHOL test can be used to assess the metabolic state of lipids, and to aid in detection of hyperlipidemia or as a screening test for hypothyroidism and hyperadrenocorticism.

Method:

TRIG

TRIG is determined enzymatically. Lipase converts the Triglycerides to Glycerol and Fatty Acids. In a subsequent step, Glycerol Kinase converts Glycerol into Glycerol Phosphate, which is oxidized, producing Dihydroxyacetone Phosphate and Peroxide (H_2O_2) in the process. The Peroxidase reaction with H_2O_2 results in the production of a wine-red colored product that has an absorbance maximum at 510 nm. The absorbance is proportional to the TRIG concentration.

$$\text{Triglycerides} + H_2O \xrightarrow{\text{LPL}} \text{Glycerol} + \text{Fatty Acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow[Mg^{2+}]{\text{GK}} \text{Glycerol-3-Phosphate} + \text{ADP}$$

$$\text{Glycerol-3-Phosphate} + O_2 \xrightarrow{\text{GPO}} \text{Dihydroxyacetonphosphate} + H_2O_2$$

$$H_2O_2 + \text{4-AAP} + \text{DCHBS} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + H_2O$$

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 CHOL concentration.

$$\text{Cholesterol Esters} + H_2O \xrightarrow{\text{COE}} \text{Cholesterol} + \text{RCOOH}$$

$$\text{Cholesterol} + NAD^+ \xrightarrow{\text{CDH}} \text{Cholest-4-En-3-One} + \text{NADH} + H^+$$

3. Reagents

Major Composition:

Composition	Quantity/Panel
Cholesterol Dehydrogenase	0.36 U
Cholesterol Esterase	1.5 U
NAD	0.28 mg
4-Aminoantipyrine	0.003 mg
Magnesium chloride	0.01 mg
Adenosine 5′-triphosphate disodium salt hydrate	0.07 mg
lipoprotein lipase	0.14 U
glycerol kinase	0.04 U
glycerol-3-phosphate oxidase	0.03 U
Horseradish Peroxidase	0.56 U
3,5-Dichloro-2-hydroxybenzenesulfonic acid	0.06 mg

Reagent Storage:

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

4. Specimen Collection and Preparation

Specimen Collection:

- Specimens suitable for skyla TRIG/CHOL single assay cartridge include lithium heparinized plasma, serum and quality control materials. The plasma or serum sample

requirement is 50 µL.

- If applicable, local regulatory or standard operating procedures of your organization should be followed for the collection, preservation and handling of specimens.

Note:

1. **The centrifugation of whole blood sample should be done within 120 minutes (at room temperature) in order to prevent cellulose precipitation in the blood.**
2. **Do not use specimens containing other coagulants. That would cause an incorrect test results.**

Specimen Preparation:

- Before applying a sample to the cartridge, the specimen should be diluted with diluent. Please use the blue 50 µL pipette to transfer the 50 µL specimen (plasma or serum) into the dilution tube.
- After injecting the specimen, close the cap tightly and invert it 10 times to thoroughly mix the solution.

Note:

1. **Once the diluent spilled out from the dilution tube during handling or the insufficient liquid was observed, please don't use that dilution tube and change the new one.**
2. **Perform testing within 10 minutes after applying the sample to the cartridge (at room temperature).**

For further information in specimen collection and preparation, please refer to "skyla Analyzer Operator's Manual".

5. Test Procedures

Test Conditions:

Test should be carried out in an environment with temperatures of 10°C~32°C. Each test will take about 10 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 take the single assay cartridge out from the pouch.
2. Put the cartridge into a slot on the single assay carrier. (The single assay carrier can hold a maximum of three single assay cartridges.)

3. Put the dummy cartridges into other unused slots on the single assay carrier.
4. Use the blue 50 μL pipette to transfer the diluted specimen from the dilution tube to the single assay cartridge **twice**, totally 100 μL of the diluted specimen should be loaded into the sample port on the cartridge through 2 loads.
5. Use a lint-free tissue to clean any sample spilled on the outside of the single assay cartridge.
6. Press the "start" button on the screen to initiate testing.
7. Place the single assay carrier on the analyzer drawer, and press the "ok" button on the screen to analysis.

Note:

1. **To avoid errors in the system when reading data, never use a used single assay cartridge as a dummy cartridge.**
2. **To operate the cartridge or instrument, please wear lab gloves and other protective gear to avoid contamination by specimen.**
3. **The used cartridge, tips, tissues should be discarded as biomedical waste, and treat according to the local legal requirements.**
4. **Testing should be performed within 20 minutes after the pouch is opened.**
5. **Avoid placing unopened reagent discs in places higher than 25°C (77°F) for more than 48 hours.**
6. **If the cartridge or its package is damaged or is over the expiry date, do not use it.**

For details on the operating steps and instrument settings, please refer to "skyla Analyzer Operator's Manual".

6. Calibration

The barcode on every manufactured cartridge 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, a confirmatory test is suggested to be carried out with the automated laboratory analyzer, or to contact our technical support.
- External quality control materials can be used for the accuracy check 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)	
CHOL	Canine	110-320	mg/dL	2.85 – 8.29	mmol/L
	Feline	54-220	mg/dL	1.40 –5.70	mmol/L
TRIG	Canine	0- 100	mg/dL	0- 1.13	mmol/L
	Feline	0- 100	mg/dL	0- 1.13	mmol/L

9. Limitation

Physiological interferences in blood include hemolysis, icterus, and lipemia. For every test item, 2 levels of 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.

Test Item	Substance concentration with interferences of less than 20%			
	Hemoglobin	Bilirubin (unconjugated)	Bilirubin (conjugated)	Intralipid
CHOL	300 mg/dL	30.0 mg/dL	30.0 mg/dL	0.4%
TRIG	450 mg/dL	13.6 mg/dL	14.0 mg/dL	-

10. Performance Characteristics

Dynamic range:

The dynamic range for each test item showed is as follows.

Test Item	Dynamic Range		Dynamic Range (SI Unit)	
CHOL	50 - 540	mg/dL	1.30 – 13.99	mmol/L
TRIG	35-600	mg/dL	0.40 -6.78	mmol/L

Method Comparison:

SIMENS ADVIA 1800 was used as comparative method in the study. The tests were performed with identical clinical serum samples for the comparison.

Marker		R^2	Slope	Intercept	Sample No.	Sample Range
CHOL	Canine	0.98313	1.02307	- 3.20047	21	66-291 mg/dL
	Feline	0.98474	1.03797	- 6.23016	23	76-236 mg/dL
TRIG	Canine	0.98473	0.97836	0.68096	25	46-332 mg/dL
	Feline	0.98790	0.94371	7.65434	16	55-219 mg/dL

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

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