skyla Reagent Kit

cCOR (Canine Cortisol)

PN：901-150

For Veterinary Use Only

Rev：B

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1. Intended Use

The skyla cCOR reagent kit used with skyla Analyzer, is intended to be used for the quantitative determination of canine cortisol (COR) in plasma and serum.

Precaution/Waring

1. This product is for in vitro diagnostic use only
2. The product must not be used individually for diagnostic purpose.
3. The Reagent kit should be stored at 2-8 °C (35.6-46.4 °F).
4. Please were the gloves when performing the test.
5. Do not re-use any part of the test kit.
6. Dispose all waste in accordance with applicable national and/or local regulations.

2. Test Component

The **skyla** cCOR reagent kit consists of all chemicals build-in the analysis cartridge.

3. Principles

The skyla cCOR reagent kit is based on competitive immunoassay using horseradish peroxidase (HRP)-labeled COR against canine COR and allows to accurate determine the concentration of cCOR in the sample.

When a sample is mixed with HRP-labeled COR, COR in the sample competitive with the HRP-labeled COR to reacts with monoclonal antibody immobilized on the polystyrene beads. Unbound

sample and enzyme conjugate are then removed by wash buffer. Finally, substrate is added to react enzyme HRP and the intensity of this colorimetric is inversely proportional to the concentration of amount.

Clinical Significance:

COR is a typical glucocorticoid hormone secreted from the bundle layer of the adrenal cortex via the activation of adrenocorticotropic hormone (ACTH). It is synthesized from cholesterol through 17α-hydroxyprogesterone and is widely involved in many basic metabolisms. Plasma COR levels are highest in the morning, and the concentration decrease to about half in the evening. Pregnancy or estrogen treatment significantly elevates cortisol levels. On the other hand, severe stress may also lead to increased cortisol production.

Since COR secretion is controlled by a feedback mechanism between the hypothalamus-pituitary-adrenal cortex, COR measurements are used as a direct monitor of adrenal status and an indirect measure of pituitary hyper or hypofunction. Elevated cortisol levels might be associated with adrenal tumors, pituitary tumors, or ectopic ACTH-producing tumors. Subnormal COR concentrations may represent adrenal hypofunction or a defect in the metabolic pathway for COR biosynthesis.

4. Reagents

Major Composition:

R1 (enzyme conjugate): 35 μL

- HRP-labeled COR in phosphate buffer saline (PBS) at pH 7.4, which consisted of 138 mM NaCl, 2.7 mM KCl.

R2 (wash buffer): 115 μL

- Tris-buffered saline with Tween 20 (TBST) at pH 7.4, which consisted of 20 mM Tris, 150 mM NaCl, 0.1% Tween 20.

R3 (substrate): 70 μL

- TMB (3, 3', 5, 5'-tetramethylbenzidine) at pH 3.6

Bead (solid phase): 1 PC

- Polystyrene bead coated with monoclonal anti-cCOR antibody

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 kit. Do not use if the kit has expired.

5. Sample Preparation

- Specimens suitable for skyla cCOR reagent kit include lithium heparinized plasma, serum, and quality control materials. The requirement of sample volume for each test is 35 μL.
- If using a whole blood sample, the sample should be centrifuged with an appropriate centrifuge before the test.
- If applicable, local regulatory or standard operating procedures of your organization should be followed for the collection, preservation, and handling of specimens.
- To analysis sample immediately after collection is recommended for good test result.

Note:

1. **The centrifugation of whole blood sample should be done within 60 minutes (at room temperature) to prevent cellulose precipitation in the blood.**
2. **Do not use specimens containing other coagulants. That would cause an incorrect test result.**
3. **The lipemic sample may affect result. For good test result, if the sample is cloudy obviously, the high-speed centrifuge with force of 10,000 × g is recommended to remove the lipid layer from the supernatant before the test.**

6. Test Procedures

Reagent Cartridge Preparation

1. Tear the foil pouch from the notch on the edge and take out the analysis cartridge.

Placing the Cartridge onto the Carrier

2. Rotate the round slot of the carrier to the position to aim the blue mark to the carrier center.
3. Align the blue mark of the Cartridge-E to the blue mark of the slot to insert the cartridge into the slot along the indicated direction.
4. Press down the cartridge until the “click” sound is produced.

Sample to the Cartridge

5. Get the centrifugal sample.
6. Use the pipette to draw 35 μL sample from the tube.

7. Inject 35uL sample into the cartridge.

Performing a Test

8. Choose the Immuno bay and press “Start” icon to launch a test.
9. The Patient ID and Species must be identified before you can open the drawer to place Cartridge with the carrier on drawer for analysis.
10. Put the carrier on the tray then press “OK” to begin the analysis.

Note:

1. **To operate the cartridge or instrument, please wear lab gloves and other protective gear to avoid contamination by specimen.**
2. **The used kit, tips, tissues should be discarded as biomedical waste, and treat according to the local legal requirements.**
3. **Please perform the test immediately once the reagent kit is taken out from storage.**
4. **Reagent kit retrieved from 2-8°C storage can be directly used without warming-up. 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 setting, please refer to “skyla Analyzer Operator’s Manual”.

7. Calibration

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

8. 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 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 follow, 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.

9. 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.

Pre-ACTH		
< 2.0 µg/dL		Must evaluate in conjunction with post-ACTH result. If both results are <2 µg/dL, results are consistent with hypoadrenocorticism.
2.0 – 6.0 µg/dL		Normal
> 22 µg/dL		Consistent with Cushing's syndrome; perform HDDST.
Post-ACTH		
< 2.0 µg/dL		Must evaluate in conjunction with pre-ACTH result. If both results are <2 µg/dL, results are consistent with hypoadrenocorticism.
2.0 – 5.9 µg/dL		Inconclusive
6.0 – 18 µg/dL		Normal
18.1 – 22.0 µg/dL		Equivocal; Cushing's syndrome possible
> 22 µg/dL		Consistent with Cushing's syndrome; perform HDDST to discriminate between PDH and ATH.
4-Hrs	8-Hrs	LDDST
-	<1 µg/dL	Normal
1–1.5 µg/dL	1–1.5 µg/dL	Inconclusive, consider repeating in 8–12 weeks
>1.5 µg/dL and >50% of baseline	>1.5 µg/dL and >50% of baseline	Consistent with Cushing's syndrome; perform HDDST to discriminate between PDH and ATH.
<1.5 µg/dL or <50% of baseline	>1.5 µg/dL and >50% of baseline	Consistent with PDH
>1.5 µg/dL or >50% of baseline	>1.5 µg/dL and <50% of baseline	Consistent with PDH
<1.5 µg/dL or <50% of baseline	>1.5 µg/dL and <50% of baseline	Consistent with PDH
4-Hrs	8-Hrs	HDDST
<1.5 µg/dL or <50% of baseline	>1.5 µg/dL and >50% of baseline	Consistent with PDH
>1.5 µg/dL and >50% of baseline	<1.5 µg/dL or <50% of baseline	Consistent with PDH

<1.5 µg/dL or <50% of baseline	<1.5 µg/dL or <50% of baseline	Consistent with PDH
>1.5 µg/dL and >50% of baseline	>1.5 µg/dL and >50% of baseline	Additional testing required to differentiate PDH from ATH.

Pre-ACTH		
< 55.2 nmol/L		Must evaluate in conjunction with post-ACTH result. If both results are < 55.2 nmol/L, results are consistent with hypoadrenocorticism.
55.2 – 166 nmol/L		Normal
> 607 nmol/L		Consistent with Cushing's syndrome; perform HDDST.
Post-ACTH		
< 55.2 nmol/L		Must evaluate in conjunction with pre-ACTH result. If both results are < 55.2 nmol/L, results are consistent with hypoadrenocorticism.
55.2 – 166 nmol/L		Inconclusive
167 – 496 nmol/L		Normal
497 – 607 nmol/L		Equivocal; Cushing's syndrome possible
> 607 nmol/L		Consistent with Cushing's syndrome; perform HDDST to discriminate between PDH and ATH.
4-Hrs	8-Hrs	LDDST
-	<27.6 nmol/L	Normal
27.6–41.4 nmol/L	27.6–41.4 nmol/L	Inconclusive, consider repeating in 8–12 weeks
>41.4 nmol/L and >50% of baseline	>41.4 nmol/L and >50% of baseline	Consistent with Cushing's syndrome; perform HDDST to discriminate between PDH and ATH.
<41.4 nmol/L or <50% of baseline	>41.4 nmol/L and >50% of baseline	Consistent with PDH
>41.4 nmol/L or >50% of baseline	>41.4 nmol/L and <50% of baseline	Consistent with PDH
<41.4 nmol/L or <50% of baseline	>41.4 nmol/L and <50% of baseline	Consistent with PDH
4-Hrs	8-Hrs	HDDST
<41.4 nmol/L or <50% of baseline	>41.4 nmol/L and >50% of baseline	Consistent with PDH
>41.4 nmol/L and >50% of baseline	<41.4 nmol/L or <50% of baseline	Consistent with PDH

<41.4 nmol/L or <50% of baseline	<41.4 nmol/L or <50% of baseline	Consistent with PDH
>41.4 nmol/L and >50% of baseline	>41.4 nmol/L and >50% of baseline	Additional testing required to differentiate PDH from ATH.

10. 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	Intralipid
cCOR	400 mg/dL	18.9 mg/dL	1017 mg/dL

11. Performance Characteristics

Dynamic range:

The dynamic range of cCOR reagent kit is as follows.

Test Item	Dynamic Range	Dynamic Range (SI Unit)
cCOR	1.0 – 30.0 μg/dL	27.6 – 828 nmol/L

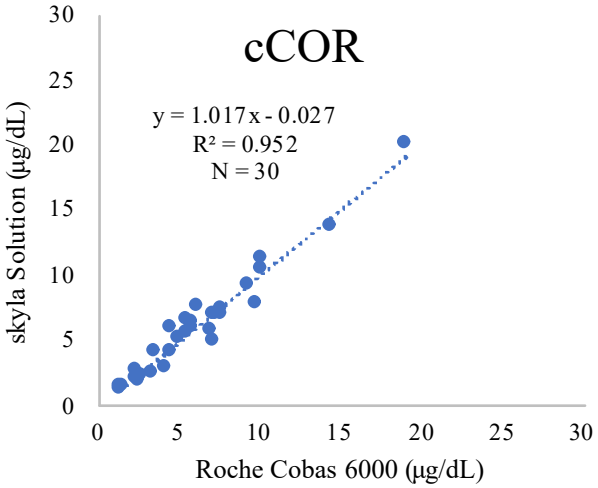
Imprecision:

Precision studies adopt control solution of high and low concentrations as test samples. Tests are performed 3 repeats a day for a total of 5 days. Results are shown in the table below.

Test Item	cCOR	
Level	Control, Low	Control, High
Unit	μg/dL	μg/dL
Mean	6.9	17.6
Std.	0.321	0.928
%CV	4.7%	5.3%

Method Comparison:

The method comparison was carried out with Roche Cobas 6000. Correlation between the different testing systems can be determined through statistical analysis. Totally 30 canine plasma samples (N=30) between 1.3 µg/dL and 20.2 µg/dL were evaluated. The regression equation was y=1.017x-0.027 and correlation coefficient R=0.952.



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

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