



Diagnostics Biochem Canada Inc.

Manufacturer of In Vitro Diagnostic Test Kits Since 1973

Chemiluminescence Immunoassay (LIA)

Cortisol

Rapid, Sensitive, Direct Microtiter Strip Immunoassay

Cat. No.: CAN-C-7010

Version: 3.0

Effective: January 8, 2008

INTENDED USE

For the direct quantitative determination of Cortisol in human serum by chemiluminescence immunoassay (LIA). For *in vitro* use only.

PRINCIPLE OF THE TEST

The principle of the following chemiluminescence immunoassay (LIA) test follows the typical competitive binding scenario. Competition occurs between an unlabeled antigen (present in standards, control and patient samples) and an enzyme-labelled antigen (conjugate) for a limited number of antibody binding sites on the microwell plate. The washing and decanting procedures remove unbound materials. After the washing step, the luminescence substrate solution is added. The relative luminescence units (RLUs) are measured on a microtiter plate luminometer. The RLU values are inversely proportional to the concentration of cortisol in the sample. A set of calibrators are used to plot a standard curve from which the amount of cortisol in patient samples and controls can be directly read.

CLINICAL APPLICATIONS

Cortisol is the most abundant circulating steroid and the major glucocorticoid secreted by the adrenal cortex. Cortisol is physiologically effective in blood pressure maintenance and anti-inflammatory activity. It is also involved in calcium absorption, gluconeogenesis as well as the secretion of gastric acid and pepsin.

Measurement of blood cortisol levels can be used as an indicator of adrenal function and the differential diagnosis of Addison's and Cushing's diseases as well as adrenal hyperplasia and carcinoma.

Most circulating cortisol is bound to cortisol binding globulin or transcortin. Therefore, the free cortisol concentration excreted in the urine is very small, and the 24-hour collection of urine is a must in order to obtain an accurate measurement of urinary cortisol. Cortisol in blood shows a diurnal rhythm with the highest levels in the morning and the lowest levels at night.

PROCEDURAL CAUTIONS AND WARNINGS

- Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
- Control materials or serum pools should be included in every run at a high and low level for assessing the reliability of results.
- When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
- In order to reduce exposure to potentially harmful substances, gloves should be worn when handling kit reagents and human specimens.
- All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
- A calibrator curve must be established for every run.
- The kit control should be included in every run and fall within established confidence limits.
- Improper procedural techniques, imprecise pipetting, incomplete washing as well as improper reagent storage may be indicated when assay values for the control do not reflect established ranges.
- The luminescence substrate solutions (A and B) are sensitive to light and should be stored in the original dark bottle away from direct sunlight.
- The assay buffer is sensitive to light and should be stored in the original dark bottle away from direct sunlight.
- When dispensing the substrate, do not use pipettes in which this liquids will come into contact with any metal parts.
- To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard and control.
- Do not mix various lot numbers of kit components within a test and do not use any component beyond the expiration date printed on the label.
- Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.

LIMITATIONS

- All the reagents within the kit are calibrated for the direct determination of Cortisol in human serum. The kit is not calibrated for the determination of Cortisol in saliva, plasma or other specimens of human or animal origin.
- Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
- Any samples or control sera containing azide or thimerosal are not compatible with this kit, as they may lead to false results.
- Only calibrator A may be used to dilute any high serum samples. The use of any other reagents may lead to false results.
- The results obtained with this kit should never be used as the sole basis for a clinical diagnosis. For example, the occurrence of heterophilic antibodies in patients regularly exposed to animals or animal products has the potential of causing interferences in immunological tests. Consequently, the clinical diagnosis should include all aspects of a patient's background including the frequency of exposure to animals/products if false results are suspected.

SAFETY CAUTIONS AND WARNINGS

Human serum that may be used in the preparation of the standards and control has been tested and found to be non-reactive for Hepatitis B surface antigen and has also been tested for the presence of antibodies to HCV and Human Immunodeficiency Virus (HIV) and found to be negative. However no test method can offer complete assurance that HIV, HCV and Hepatitis B virus or any infectious agents are absent. The reagents should be considered a potential biohazard and handled with the same precautions as applied to any blood specimen.

SPECIMEN COLLECTION AND STORAGE

Approximately 0.1 ml of serum is required per duplicate determination. Collect 4-5 ml of blood into an appropriately labelled tube and allow it to clot. Centrifuge and carefully remove the serum layer. Store at 4°C for up to 24 hours or at -10°C or lower if the analyses are to be done at a later date. Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling

CHEMICAL HAZARDS

Avoid direct contact with reagents. In case of contact, wash with plenty of water.

SPECIMEN PRETREATMENT

This assay is a direct system; no specimen pretreatment is necessary

REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

- Precision pipettes to dispense 25, 50, 100, 150 and 300 µl
- Disposable pipette tips
- Distilled or deionized water
- Plate shaker
- Microwell plate luminometer

REAGENTS PROVIDED AND PREPARATION

1. Rabbit Anti-Cortisol Antibody Coated Microwell Plate-Break Apart Wells - Ready To Use.

Contents: One 96 well (12x8) polyclonal antibody-coated microwell plate in a resealable pouch with desiccant.

Storage: Refrigerate at 2-8°C

Stability: 12 months or as indicated on label.

2. Cortisol-Horse Radish Peroxidase (HRP) Conjugate Concentrate - Requires Preparation.

Contents: Cortisol-HRP conjugate in a protein-based buffer with a non-mercury preservative.

Volume: 300 µl/vial

Storage: Refrigerate at 2-8°C

Stability: 12 months or as indicated on label.

Preparation: Dilute 1:100 in assay buffer before use (eg. 20 µl of HRP in 2 ml of assay buffer). If the whole plate is to be used dilute 120 µl of HRP in 12ml of assay buffer. Discard any that is left over.

3. Cortisol Calibrators - Ready To Use.

Contents: Seven vials containing Cortisol in a human serum-based buffer with a non-mercury preservative. Prepared by spiking serum with a defined quantity of Cortisol.

*Listed below are approximate concentrations, please refer to vial labels for exact concentrations.

Calibrator	Concentration	Volume/Vial
Calibrator A	0 µg/dL	1.0 mL
Calibrator B	0.5 µg/dL	0.3 mL
Calibrator C	2 µg/dL	0.3 mL
Calibrator D	5 µg/dL	0.3 mL
Calibrator E	10 µg/dL	0.3 mL
Calibrator F	30 µg/dL	0.3 mL
Calibrator G	60 µg/dL	0.3 mL

Storage: Refrigerate at 2-8°C

Stability: 12 months in unopened vials or as indicated on label. Once opened, the standards should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

4. Control - Ready To Use.

Contents: One vial containing Cortisol in a human serum-based buffer with a non-mercury preservative. Prepared by spiking buffer with a defined quantity of Cortisol. Refer to vial label for expected value and acceptable range.

Volume: 0.3 ml/vial

Storage: Refrigerate at 2-8°C

Stability: 12 months in unopened vial or as indicated on label. Once opened, the control should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

5. Wash Buffer Concentrate - Requires Preparation.

Contents: One bottle containing buffer with a non-ionic detergent and a non-mercury preservative.

Volume: 50 ml/bottle

Storage: Refrigerate at 2-8°C

Stability: 12 months or as indicated on label.

Preparation: Dilute 1:10 in distilled or deionized water before use. If the whole plate is to be used dilute 50 ml of the wash buffer concentrate in 450 ml of water.

6. Assay Buffer - Ready To Use.

Contents: One vial containing a protein-based buffer with a non-mercury preservative.

Volume: 15 ml/vial

Storage: Refrigerate at 2-8°C

Stability: 12 months or as indicated on label.

7. Chemiluminescence Substrate Reagent A - Requires Preparation.

Contents: One bottle containing luminol enhancer.

Volume: 1 ml/bottle

Storage: Refrigerate at 2-8°C

Stability: 12 months or as indicated on label.

Preparation: See below.

8. Chemiluminescence Substrate Reagent B - Requires Preparation.

Contents: One vial containing peroxide solution.

Volume: 1 ml/vial

Storage: Refrigerate at 2-8°C

Stability: 12 months or as indicated on label.

Preparation: See below.

9. Chemiluminescence Substrate Reagent C - Requires Preparation.

Contents: One vial containing buffer with a non-mercury preservative.

Volume: 15 ml/vial

Storage: Refrigerate at 2-8°C

Stability: 12 months or as indicated on label.

Preparation: See below.

Preparation of Working Substrate Solution:

Mix 1 part of the chemiluminescence substrate reagent A with 1 part of reagent B and dilute this mixture 1:5 with reagent C. This gives the ready to use substrate solution. Prepare fresh for each use.

If the whole plate is to be used prepare working substrate solution as follows:

Combine 1 ml of reagent A with 1 ml of reagent B. To the 2 ml of this mixture add 10 ml of reagent C. Total volume=12 ml of working substrate solution.

Stability: Working substrate solution is stable for 24 hours at room temperature.

ASSAY PROCEDURESpecimen Pretreatment: *None.***Important Notes:**

- All reagents must reach room temperature before use.
- Once the procedure has been started, all steps should be completed without interruption to ensure equal elapsed time for each pipetting step.
- The washing procedure influences the precision markedly; it is essential to ensure the washing is effective and thorough.

- Remove the required number of microwell strips. Reseal the bag and return any unused strips to the refrigerator.
- Pipette 25 µl of each calibrator, control and specimen sample into correspondingly labelled wells in duplicate.
- Pipette 100 µl of the conjugate working solution into each well (We recommend using a multichannel pipette).
- Incubate on a plate shaker (approximately 200 rpm) for 1 hour at room temperature.
- Wash the wells 5 times with 300 µl of diluted wash buffer per well and tap the plate firmly against absorbent paper to ensure that it is dry (The use of a washer is recommended).
- Pipette 100 µl of chemiluminescence working substrate solution into each well (We recommend using a multichannel pipette).
- Shake for 5 seconds. Incubate for 15 minutes at room temperature without shaking.
- Measure the RLU in each well on a microplate luminometer within 20 minutes after addition of the substrate.

CALCULATIONS

- Calculate the mean RLU of each calibrator duplicate.
- Draw a calibrator curve on semi-log paper with the mean RLU on the Y-axis and the calibrator concentrations on the X-axis. If immunoassay software is being used, a 4-parameter curve is recommended.
- Calculate the mean RLU of each unknown duplicate.
- Read the values of the unknowns directly off the calibrator curve.
- If a sample reads more than 1000 pg/ml then dilute it with calibrator A at a dilution of no more than 1:8. The result obtained should be multiplied by the dilution factor.

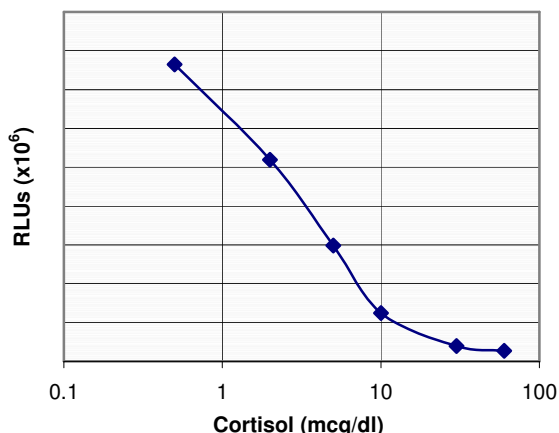
TYPICAL TABULATED DATA**

Calibrator	RLU 1 x 10 ³	RLU 2 x 10 ³	Mean RLU x 10 ³	RLU/RLU _{MAX} (%)
A, 0 µg/ml	2812	2900	2856	100
B, 0.5 µg/ml	2375	2372	2374	83.1
C, 2 µg/ml	1604	1532	1568	54.9
D, 5 µg/ml	911	854	882	30.9
E, 10 µg/ml	213	220	216	7.6
F, 30 µg/ml	110	102	106	3.7
G, 60 µg/ml	91	87	89	3.1

** It is recommended to use the RLU/RLU_{MAX} values for comparative purposes since luminometers vary considerably between manufacturers. Results from different luminometers will show quite different RLU values, however, the RLU/RLU_{MAX} values remain consistent.

TYPICAL CALIBRATOR CURVE

Sample curve only. Do not use to calculate results.

**PERFORMANCE CHARACTERISTICS SENSITIVITY**

The lower detection limit is calculated from the standard curve by determining the resulting concentration of the mean RLU of Calibrator A (based on 10 replicate analyses) minus 2 SD. Therefore, the sensitivity of the dbc Direct Cortisol LIA kit is 0.15 µg/dL.

SPECIFICITY (CROSS REACTIVITY)

The following compounds were tested for cross-reactivity with the Direct Cortisol LIA kit with cortisol cross-reacting at 100%.

Steroid	%Cross Reactivity
Cortisol	100
Prednisolone	13.6
Corticosterone	7.6
Deoxycorticosterone	7.2
Progesterone	7.2
Cortisone	6.2
Deoxycortisol	5.6
Pednisone	5.6
Dexamethasone	1.6

No cross reaction was detected with DHEAS and Tetrahydrocortisone. Please note that there is an observed cross-reactivity of 13.6% with prednisolone. Since prednisone is converted to prednisolone in vivo, caution must be exercised when assaying the cortisol levels of patients undergoing either therapy.

INTRA-ASSAY PRECISION

Three samples were assayed sixteen times each on the same calibrator curve. The results (in µg/dL) are tabulated below:

Sample	Mean	SD	CV%
1	1.01	0.10	9.9
2	10.44	0.36	3.4
3	29.60	2.82	9.5

INTER-ASSAY PRECISION

Three samples were assayed ten times over a period of two weeks. The results (in µg/dL) are tabulated below:

Sample	Mean	SD	CV%
1	1.39	0.13	9.4
2	15.01	0.74	4.9
3	38.18	2.62	6.9

RECOVERY

Samples were Spiked by adding different Cortisol standards at 1:1 volume to two patient serum samples. The results (in µg/dL) are tabulated below:

Sample	Obs.Result	Exp.Result	Recovery%
1 Unspiked	5.8	-	-
+ 10 µg/dL standard	7.9	7.9	100.0
+ 30 µg/dL standard	18.3	17.9	102.2
+ 60 µg/dL standard	23.5	32.9	71.4
2 Unspiked	9.6	-	-
+ 2 µg/dL standard	5.7	5.8	98.6
+ 5 µg/dL standard	6.5	7.3	109.9
+ 10 µg/dL standard	10.1	9.8	103.1

LINEARITY

Three patient serum samples were diluted with calibrator A. The results (in µg/dL) are tabulated below:

Sample	Obs.Result	Exp.Result	Recovery%
1	6.1	-	-
1:2	2.8	3.1	90.3
1:4	1.6	1.6	100.0
1:8	0.9	0.8	112.5
2	7.6	-	-
1:2	3.6	3.8	94.7
1:4	2.0	1.9	105.3
1:8	1.1	1.0	110.0
3	24.8	-	-
1:2	10.5	12.4	84.7
1:4	5.3	6.2	85.5
1:8	2.8	3.1	90.3

EXPECTED NORMAL VALUES-SERUM

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values.

Group	Mean (µg/dL)	Range (µg/dL)
Males and Females – AM	15.59	3.95-27.23
Males and Females – PM	5.93	1.45-10.41

REFERENCES

- Brock P., et al., Clinical Chemistry 24/9:1595, 1978.
- Morris R., Annals of Clinical Biochemistry 15:178, 1978.
- Silver A.C., et al., Clinical Chemistry 29:1869, 1983.
- Vecsei P., et al., Experimentia 28:1104, 1972.
- Abraham G.E. et al., Anal Lett. 5:757, 1972.
- Gomez-Sanchez C., et al., J. Lab. Clin. Med 89:902, 1977.
- Demeris L.M., et al., Clin. Biochem. 10:104, 1977.
- Poland R.E., et al., Life Sci. 30:177, 1982.
- Peters J.R., et al., Clin Endocrinol. 17:583, 1982.
- Papanicolaou, D.A. et al J. Clin Endocrinol Metab 87(10):4515-4521.
- Check, J.H., et al, Falsely elevated steroidal assay levels related to heterophile antibodies against various animal species. Gynecol Obstet Invest 40:139-140, 1995.

OTHER RELATED DBC KITS

Also available from stock are the following dbc kits:

dbc Direct Cortisol ELISA Kit, Cat. No.:CAN-C-270

Please contact us if you require any further information.

CONTACT INFORMATION

Diagnostics Biochem Canada Inc.
1020 Hargrieve Road
London, Ontario, Canada, N6E 1P5
Tel/Fax: (519) 681 8731, E-mail:dbc@dbc-labs.com
Internet: www.dbc-labs.com

ISO 9001:2000 and ISO 13485 Certified