***Diagnostics Biochem Canada Inc.***
*Manufacturer of In Vitro Diagnostic Test Kits Since 1973*

# Chemiluminescence Immunoassay (LIA)
# Aldosterone

*Rapid, Sensitive, Direct Microtiter Strip Immunoassay*

*Cat. No.: CAN-ALD-6000-2* *Version: 2.1*
*Effective: March 30, 2007*

### *INTENDED USE*
For the direct quantitative determination of Aldosterone in human serum by chemiluminescence immunoassay (LIA). Hydrolysis is necessary for the determination of Aldosterone in urine
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 aldosterone in the sample. A set of calibrators are used to plot a standard curve from which the amount of aldosterone in patient serum samples and controls can be directly read.

### *CLINICAL APPLICATIONS*
Aldosterone is a potent mineral corticoid whose synthesis and release are controlled by the renin-angiotensin system of the body. Aldosterone promotes the reabsorption of sodium in the distal tubules of the kidney resulting in potassium secretion along with sodium retention, which controls the circulating blood volume. Chronic overproduction and secretion of aldosterone leads to hypertension. Measurement of aldosterone levels in serum in conjunction with plasma renin levels can be used to differentiate between primary and secondary aldosteronism.

| Condition | Serum Aldosterone | Plasma Renin |
|---|---|---|
| Primary Aldosteronism | High | Low |
| Secondary Aldosteronism | High | High |

The measurement of aldosterone in concert with selective suppression and stimulation tests can be used to further differentiate primary aldosteronism into two basic types:
1.Primary aldosteronism caused by an adenoma of one or both adrenals.
2.Primary aldosteronism caused by adrenal hyperplasia.
This differentiation is vital in the treatment and management of the disease. The adrenal adenomas respond well to surgery whereas hyperplastic disease of the adrenals is generally better managed medically. In summary, the precise and accurate measurement of serum aldosterone can be an important adjunct to a diagnostic laboratory battery for the differential diagnosis of hypertensive disease.

### *PROCEDURAL CAUTIONS AND WARNINGS*
1. 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.
2. Users are recommended to include their own control materials or serum pools in every run for assessing the reliability of results.
3. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
4. In order to reduce exposure to potentially harmful substances, gloves should be worn when handling kit reagents and human specimens.
5. 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.
6. A calibrator curve must be established for every run.
7. The kit control should be included in every run and fall within established confidence limits.
8. 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.
9. The luminescence substrate solutions (A and B) are sensitive to light and should be stored in the original dark bottle away from direct sunlight.
be properly stored.
10. The assay buffer is sensitive to light and should be stored in the original dark bottle away from direct sunlight.
12. When dispensing the substrate, do not use pipettes in which this liquids will come into contact with any metal parts.
13. To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard and control.
14. 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.
15. Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.

### *LIMITATIONS*
1. All the reagents within the kit are calibrated for the direct determination of aldosterone in human serum. The kit is not calibrated for the determination of aldosterone in saliva, plasma or other specimens of human or animal origin.
2. Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
3. Any samples or control sera containing azide or thimerosal are not compatible with this kit, as they may lead to false results.
4. Only calibrator A may be used to dilute any high serum samples. The use of any other reagents may lead to false results.
5. 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*
### *POTENTIAL BIOHAZARDOUS MATERIAL*
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.

### *CHEMICAL HAZARDS*
Avoid direct contact with reagents. In case of contact, wash with plenty of water.

### *SPECIMEN COLLECTION AND STORAGE*
Serum: Approximately 0.2 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.

Urine: Approximately 1 ml of urine is required per duplicate determination. Collect 24-hour urine into a specimen collection container. 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.

### *SERUM PRETREATMENT*
No specimen pretreatment is necessary.

### *URINE PRETREATMENT*
1. Label one glass or polypropylene tube for each urine sample.
2. Pipet 1 mL of each urine sample into an appropriate tube.
* If the sample is cloudy, first centrifuge the urine and work with the supernatant.
3. Hydrolysis: Add 0.1 mL of 3.2 N HCl (not supplied) to every tube. Cap securely and heat for 1 hour at 60°C in the dark.
* 3.2 N HCl can be made by adding 1 mL of concentrated HCl (12N) to 2.75 mL distilled water.
4. Neutralization: Add 0.1 mL of 3.2 N NaOH to every tube and mix gently and thoroughly.
* 3.2 N NaOH can be made by dissolving 1.28 grams of NaOH pellets into 10 mL distilled water.
5. Dilution: Dilute the neutralized samples 1:50 with calibrator A.

### *REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED*
1. Precision pipettes to dispense 50, 100, 150 and 300 µl
2. Disposable pipette tips
3. Distilled or deionized water
4. 3.2 N HCl and 3.2 N NaOH (for urine analysis)
5. Glass or polypropylene tubes (for urine analysis)
6. Water bath (for urine analysis)
7. Plate shaker
8. Microwell plate luminometer

### *REAGENTS PROVIDED AND PREPARATION*
**1. Rabbit Anti-Aldosterone 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. Aldosterone-Biotin : Avidin-Horse Radish Peroxidase (HRP) Conjugate Concentrate** - Requires Preparation.
Contents: Aldosterone-biotin and avidin-HRP conjugates in a protein-based buffer with a non-mercury preservative.
Volume: 200 µl/vial
Storage: Refrigerate at 2-8°C
Stability: 12 months or as indicated on label.
Preparation: Dilute the aldosterone-biotin:avidin-HRP concentrate 1:100 in assay buffer before use. 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. Aldosterone Calibrators** - Ready To Use.
Contents: Six vials containing aldosterone in a protein-based buffer with a non-mercury preservative. Prepared by spiking matrix with a defined quantity of aldosterone.
*Listed below are approximate concentrations, please refer to vial labels for exact concentrations.

| Calibrator | Concentration | Volume/Vial |
|---|---|---|
| Calibrator A | 0 pg/ml | 2.0 ml |
| Calibrator B | 20 pg/ml | 0.5 ml |
| Calibrator C | 80 pg/ml | 0.5 ml |
| Calibrator D | 300 pg/ml | 0.5 ml |
| Calibrator E | 800 pg/ml | 0.5 ml |
| Calibrator F | 2000 pg/ml | 0.5 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 aldosterone in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with a defined quantity of aldosterone. Refer to vial label for expected value and acceptable range.
Volume: 0.5 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.

**9. Cheminluminescence Substrate Reagent A -** Requires Preparation.
Volume: 1.0 ml/bottle
Storage: Refrigerate at 2-8°C
Stability: as indicated on label.
Preparation: See below.

**10. Cheminluminescence Substrate Reagent B** - Requires Preparation.
Volume: 1.0 ml/vial
Storage: Refrigerate at 2-8°C
Stability: as indicated on label.
Preparation: See below.

**11. Cheminluminescence Substrate Reagent C** - Requires Preparation.
Contents: One vial containing buffer with a non-mercury preservative.
Volume: 16 ml/vial
Storage: Refrigerate at 2-8°C
Stability: as indicated on label.
Preparation: See below.

**Preparation of Working Substrate Solution:**
In a clean plastic container (glass is not suitable) mix 1 part of the substrate reagent A with 1 part of reagent B and 20 parts of substrate reagent C. This gives the ready to use substrate solution. If the whole plate is to be used prepare working substrate solution as follows: Combine 0.8 ml of reagent A with 0.8 ml of reagent B and 16 ml of reagent C. It is suggested to wait at least 2 minutes prior to use after preparation of the working substrate solution.The working substrate solution is stable for up to 2 hours at room temperature. Discard the leftovers.

## *ASSAY PROCEDURE*
Specimen Pretreatment:
***Serum: None.***
***Urine: Hydrolysis, Neutralization and Dilution***
*(see detailed instructions under Urine Pretreatment)*

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

1. **Remove the required number of microwell strips. Reseal the bag and return any unused strips to the refrigerator.**
2. **Pipette 50 µl of each calibrator, control and specimen sample into correspondingly labelled wells in duplicate.**
3. **Pipette 100 µl of the conjugate working solution into each well (We recommend using a multichannel pipette).**
4. **Incubate on a plate shaker (approximately 200 rpm) for 60 minutes at room temperature.**
5. **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).**
6. **Pipette 150 µl of chemiluminescence working substrate solution into each well (We recommend using a multichannel pipette).**
7. **Incubate without shaking for 10 minutes at room temperature.**
8. **Measure the RLUs in each well on a microplate luminometer within 20 minutes after addition of the substrate.**

## *CALCULATIONS*
1. Calculate the mean RLU of each calibrator duplicate.
2. Draw a calibrator curve on semi-log paper with the mean RLUs on the Y-axis and the calibrator concentrations on the X-axis. If immunoassay software is being used, a 4-parameter curve is recommended.
3. Calculate the mean RLU of each unknown duplicate.
4. Read the values of the serum samples directly off the calibrator curve. If a serum sample reads more than 2000 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
5. Read the values of the urine samples directly off the curve and multiply by a factor of 60 (the original urine samples are diluted 1-in-1.2 and 1-in-50, see the urine pretreatment). Next, multiply by the volume of collected 24-hour urine (in litres). Finally, divide this figure by 1000 to obtain values in µg/24 hour If a urine sample reads more than 2000 pg/ml then dilute it with the calibrator A at a dilution of no more than 1:2 (from the original 1:50 dilution). 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 pg/ml | 1561 | 1445 | 1503 | 100 |
| B, 20 pg/ml | 1085 | 1097 | 1091 | 73 |
| C, 80 pg/ml | 593 | 593 | 593 | 40 |
| D, 300 pg/ml | 193 | 194 | 194 | 13 |
| E, 800 pg/ml | 44 | 43 | 44 | 3 |
| F, 2000 pg/ml | 7 | 5 | 6 | 0.4 |

**- 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.

## *EXPECTED NORMAL VALUES*
As for all clinical assays each laboratory should collect data and establish their own range of expected normal values. The results of an expected range study with apparently normal healthy subjects yielded the following results (all values are reported in pg/ml):

| | |
|---|---|
| **Group:** | Unrestricted salt intake, seated position |
| **Subjects:** | n = 54 |
| **Mean:** | 105 pg/ml |
| **Expected Range (As central 95 pencentile ):** | 25-315 pg/ml |

## *REFERENCE NORMAL VALUES-URINE*
As for all clinical assays each laboratory should collect data and establish their own range of expected normal values.

| Group | Range (µg/24 hr) |
|---|---|
| Normal Salt Intake | 5-19 |

Wilson, J.D. and Foster, D.W. Williams Textbook of Endocrinology 8th Edition. W.B. Saunders Company, London. p 582, 1992.

## *PERFORMANCE CHARACTERISTICS*
### *SENSITIVITY*
The lower detection limit is calculated from the standard curve by determining the resulting concentration of the mean OD of Calibrator A (based on 10 replicate analyses) minus 2 SD. Therefore, the sensitivity of the dbc Direct Aldosterone ELISA kit is **4.24 pg/ml.**

### *SPECIFICITY (CROSS REACTIVITY)*
The following compounds were tested for cross-reactivity with the Direct Aldosterone ELISA kit with aldosterone cross-reacting at 100%.

| Steroid | %Cross Reactivity |
|---|---|
| Aldosterone | 100 |
| 11-Deoxycorticosterone | 1.1 |

The following steroids were tested but cross-reacted at less than 0.001%: Androsterone, Cortisone, 11-Deoxycortisol, 21-Deoxycortisol, Dihydrotestosterone, Estradiol, Estriol, Estrone and Testosterone.

### *INTRA-ASSAY PRECISION*
Three samples were assayed ten times each on the same calibrator curve. The results (in pg/ml) are tabulated below:

| Sample | Mean | SD | CV% |
|---|---|---|---|
| 1 | 113.23 | 6.23 | 5.5 |
| 2 | 342.15 | 16.08 | 4.7 |
| 3 | 804.59 | 43.45 | 5.4 |

### *INTER-ASSAY PRECISION*
Three samples were assayed ten times over a period of four weeks. The results (in pg/ml) are tabulated below:

| Sample | Mean | SD | CV% |
|---|---|---|---|
| 1 | 105.65 | 6.76 | 6.7 |
| 2 | 332.87 | 21.97 | 6.6 |
| 3 | 785.61 | 63.63 | 8.1 |

### *RECOVERY*
Spiked samples were prepared by adding defined amounts of aldosterone to three patient serum samples. The results (in pg/ml) are tabulated below:

| Sample | Obs.Result | Exp.Result | Recovery% |
|---|---|---|---|
| 1 | | | |
| Unspiked | 60.53 | - | - |
| +300(10:1) | 73.86 | 82.30 | 89.74 |
| +300(10:2) | 99.65 | 100.44 | 99.21 |
| +2000(10:2) | 336.61 | 383.78 | 87.71 |
| 2 | | | |
| Unspiked | 120.24 | - | - |
| +300(10:1) | 123.91 | 136.58 | 90.72 |
| +300(10:2) | 132.58 | 150.20 | 88.27 |
| +2000(10:2) | 376.87 | 433.53 | 86.93 |
| 3 | | | |
| Unspiked | 153.12 | - | - |
| +300(10:1) | 188.36 | 166.47 | 113.15 |
| +300(10:2) | 174.88 | 177.60 | 98.47 |
| +2000(10:2) | 471.35 | 460.93 | 102.26 |

### *LINEARITY*
Two patient serum samples were diluted with calibrator A. The results (in pg/ml) are tabulated below:

| Sample | Obs.Result | Exp.Result | Recovery% |
|---|---|---|---|
| 1 | 336.61 | - | - |
| 1:2 | 182.18 | 168.31 | 108.24 |
| 1:4 | 82.08 | 84.15 | 97.54 |
| 1:8 | 37.23 | 42.08 | 88.47 |
| 2 | 376.87 | - | - |
| 1:2 | 226.39 | 188.44 | 120.14 |
| 1:4 | 101.39 | 94.22 | 107.61 |
| 1:8 | 47.26 | 47.11 | 100.32 |
| 3 | 471.35 | - | - |
| 1:2 | 279.45 | 235.68 | 118.57 |
| 1:4 | 115.04 | 117.84 | 97.62 |
| 1:8 | 53.13 | 58.92 | 90.17 |

## *REFERENCES*

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## *OTHER RELATED DBC KITS*
Also availablefrom stock are the following dbc kits:

- dbc Direct Aldosterone ELISA Kit, Cat. No.:CAN-ALD-450

Please contact us if you require any further information.

## *CONTACT INFORMATION*

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