EAGLE BIOSCIENCES

5α-ANDROSTANE-3α, 17β-DIOL GLUCURONINDE (3α-DIOL G) LIA

Catalog Number:
5AA31-L01 (1 x 96 wells)
For Research Use Only. Not for use in diagnostic procedures.
v. 1.0

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INTENDED USE

The Eagle Biosciences 5α-Androstane-3α, 17β-Diol Glucuroninde (3α-Diol G) LIA is for the direct quantitative determination of 5α-androstane-3α, 17β-diol glucuronide (3α-Diol G) in human serum by a chemiluminescence immunoassay (LIA). The Eagle Biosciences 5α-Androstane-3α, 17β-Diol Glucuroninde (3α-Diol G) LIA for research use only and not to be used in diagnostic procedures.

INTRODUCTION

5α-androstane-3α, 17β-diol glucuronide is a C19 steroid and is either abbreviated as 3α-Diol G, 5α diol G or simply, α diol G. It is produced mainly as a metabolite of testosterone and dihydrotestosterone (DHT). It is largely produced in target peripheral tissues such as the skin, especially around hair follicles. The stimulation by large amounts of 3α-Diol G leads to excessive hair formation, notably where hair is not normally present in women. In recent years the interest in the measurement of this steroid has increased among clinical investigators studying women suffering from idiopathic hirsutism. Among the steroids known to be precursors for 3α-Diol G are dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAS), dihydrotestosterone (DHT), androstenedione and testosterone. Only 3α-Diol G has been shown to increase with hirsutism and decrease with treatment. This correlation has also been demonstrated in patients with polycystic ovarian syndrome (PCO). 3 α-Diol G determinations have therefore proved to be a useful indicator in a variety of ways including monitoring the progress of treatment of idiopathic hirsutism and women with PCO. Furthermore, diabetic patients (both men and women) under cyclosporine A therapy have shown increased 3 α-Diol G levels, a s ide effect resulting in the appearance of hair in previously hairless areas.

PRINCIPLE OF THE ASSAY

The principle of the following chemiluminescence immunoassay (LIA) test follows the typical competitive binding scenario. Competition occurs between an unlabelled antigen (present in standards, controls and patient samples) and an enzyme-labelled antigen (conjugate) for a limited number of antibody binding sites on the microplate. 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 3α-Diol G in the sample. A set of calibrators are used to plot a standard curve from which the amount of 3α-diol G in patient samples and controls can be directly read.

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. Control materials or serum pools should be included in every run at a high and low level 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 controls 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 controls 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.
10. The assay buffer is sensitive to light and should be stored in the original dark bottle away from direct sunlight.
11. When dispensing the substrate and stopping solution, do not use pipettes in which these liquids will come into contact with any metal parts.
12. To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard and control.
13. 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.
14. 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 determination of 3α-Diol G in human serum. The kit is not calibrated for the determination of 3α-Diol G 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 m ay be used to dilute any high serum samples. The use of any other reagent may lead to false results.
5. This kit is intended for research use only and should not be used in diagnostic procedures.

SAFETY CAUTIONS AND WARNINGS
POTENTIAL BIOHAZARDOUS MATERIAL

Human serum that may be used in the preparation of the standards and controls 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. No test method however, 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 contact with reagents containing TMB, hydrogen peroxide and sulfuric acid. If contacted with any of these reagents, wash with plenty of water. TMB is a suspected carcinogen.

SPECIMEN COLLECTION AND STORAGE

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. Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.

SPECIMEN PRETREATMENT

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

REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

1. Precision pipettes to dispense 25, 50, 100, 150 and 300 µL
2. Disposable pipette tips
3. Distilled or deionized water
4. Plate shaker
5. Microplate reader with a filter set at 450 nm and an upper OD limit of 3.0 or greater* (see assay procedure step 10)

REAGENTS PROVIDED

1. Rabbit Anti-3α-Diol G Antibody-Coated Break-Apart Well Microplate — Ready To Use
   Contents: One 96-well (12x8) polyclonal antibody-coated microplate in a resealable pouch with desiccant.
   Storage: Refrigerate at 2–8°C
   Stability: 12 months or as indicated on label.

2. 3α-Diol G-Horseradish Peroxidase (HRP) Conjugate Concentrate — Requires Preparation X50
   Contents: 3α-Diol G-HRP conjugate in a protein-based buffer with a non-mercury preservative.
   Volume: 0.3 mL/vial
   Storage: Refrigerate at 2–8°C
   Stability: 12 months or as indicated on label.
   Preparation of conjugate working solution: Dilute conjugate concentrate 1:50 in assay buffer before use (e.g.: 40 µL of conjugate concentrate in 2 mL of assay buffer). If the whole plate is to be used dilute 240 µL of conjugate concentrate in 12 mL of assay buffer. Discard any that is left over.

3. 3α-Diol G Calibrators — Ready To Use
   Contents: Six vials containing 3α-Diol G in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with a defined quantity of 3α-Diol G.
   * Listed below are approximate concentrations, please refer to bottle labels for exact concentrations.

Calibrator	Concentration	Volume
Calibrator A	0 ng/mL	2.0 mL
Calibrator B	0.25 ng/mL	0.6 mL
Calibrator C	1 ng/mL	0.6 mL
Calibrator D	3 ng/mL	0.6 mL
Calibrator E	10 ng/mL	0.6 mL
Calibrator F	50 ng/mL	0.6 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. Controls — Ready To Use
   - Contents: Two vials containing 3α-Diol G in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with a defined quantity of 3α-Diol G. Refer to vial label for expected value and acceptable range.
   - Volume: 0.6 mL/vial
   - Storage: Refrigerate at 2–8°C
   - Stability: 12 months in unopened vial or as indicated on label. Once opened, the controls should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

5. Wash Buffer Concentrate – Requires Preparation x10
   - 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 one 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 bottle containing a protein-based buffer with a non-mercury preservative.
   - Volume: 15 mL/bottle
   - Storage: Refrigerate at 2–8°C
   - Stability: 12 months or as indicated on label.

7. LIA Substrate Reagent A – Requires Preparation
   - Contents: One vial containing luminol enhancer.
   - Volume: 1 mL/vial
   - Storage: Refrigerate at 2–8°C
   - Stability: 12 months or as indicated on label.
   - Preparation: See preparation of LIA working substrate solution.

8. LIA Substrate Reagent B – Requires Preparation
   - Contents: One vial containing peroxide solution.
   - Volume: 2 mL/vial
   - Storage: Refrigerate at 2–8°C
   - Stability: 12 months or as indicated on label.
   - Preparation: See preparation of LIA working substrate solution.

9. LIA Substrate Reagent C – Requires Preparation
   - Contents: One bottle containing buffer with a non-mercury preservative.
   - Volume: 15 mL/bottle
   - Storage: Refrigerate at 2–8°C
   - Stability: 12 months or as indicated on label.
   - Preparation: See preparation of LIA working substrate solution.

PREPARATION OF LIA WORKING SUBSTRATE SOLUTION

Mix 1 part of LIA substrate reagent A with 2 parts of LIA substrate reagent B and dilute this mixture 1:3.33 with LIA substrate 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 LIA substrate reagent A with 2 m L of LIA substrate reagent B. To the 3 m L of this mixture add 10 mL of LIA substrate reagent C. Total volume = 13 mL of working substrate solution. Stability: Working substrate solution is stable for 24 hours at room temperature.

ASSAY PROCEDURE

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.

1. Prepare working solutions of the conjugate, wash buffer and LIA substrate (refer to reagents provided and preparation section).
2. Remove the required number of well strips. Reseal the bag and return any unused strips to the refrigerator.
3. Pipette 50 µL of each calibrator, control and specimen sample into correspondingly labelled wells in duplicate.
4. Pipette 100 µL of the conjugate working solution into each well. (We recommend using a multichannel pipette.)
5. Incubate on a plate shaker (approximately 200 rpm) for 30 minutes at room temperature.
6. 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.)
7. Pipette 100 µL of LIA working substrate solution into each well. (We recommend using a multichannel pipette.)
8. Shake for 5 seconds. Incubate for 10–30 minutes at room temperature without shaking.
9. Measure the RLUs in each well on a microplate luminometer.

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 unknowns directly off the calibrator curve.
5. If a sample reads more than 50 ng/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.

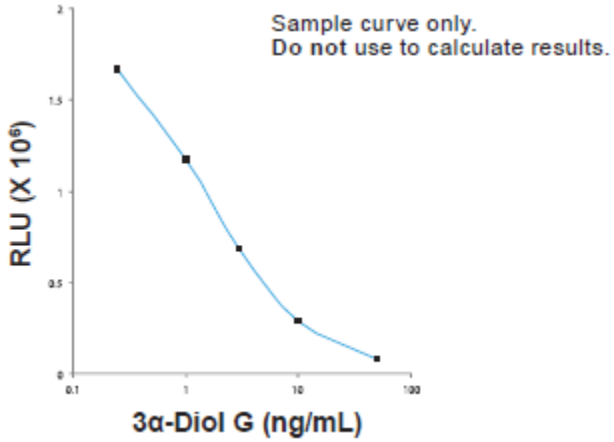
TYPICAL TABULATED DATA

Sample data only. Do not use to calculate results.

Calibrator	RLU 1 x 10^3	RLU 2 x 10^3	Mean RLU x 10^3	RLU/RLU_{MAX} (%)
A, 0 ng/mL	1914	1917	1916	100
B, 0.25 ng/mL	1669	1663	1671	87
C, 1 ng/mL	1181	1159	1170	61
D, 3ng/mL	693.8	677.1	685.4	36
E, 10 ng/mL	280.1	296.0	288.0	15
F, 50 ng/mL	84.84	83.32	84.01	4.3

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

TYPICAL CALIBRATOR CURVE



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 3α-Diol G LIA kit is 0.1 ng/mL.

SPECIFICITY (CROSS-REACTIVITY)

The following compounds were tested for cross-reactivity with the 3α-Diol G LIA kit with 3α-Diol G cross-reacting at 100%:

Steroid	% Cross Reactivity
3α-Diol G	100
Testosterone	0.2
Progesterone	0.16
Androstenedione	0.14
Cortisol	0.05

The following steroids were tested but cross-reacted at less than 0.01%: Corticosterone, Dehydroepiandrosterone, Dihydrotestosterone, Epiandrosterone, 17β-Estradiol and Estrone.

INTRA-ASSAY PRECISION

Three serum samples were assayed ten times each on the same calibrator curve. The results (in ng/mL) are tabulated below:

Sample	Mean	SD	CV %
1	1.21	0.10	7.96
2	8.86	0.32	3.58
3	32.23	1.95	6.06

INTER-ASSAY PRECISION

Three serum samples were assayed ten times over a period of four weeks. The results (in ng/mL) are tabulated below:

Sample	Mean	SD	CV %
1	1.57	0.10	6.4
2	10.49	0.87	8.34
3	43.19	3.81	8.82

RECOVERY

Spiked samples were prepared by adding defined amounts of 3α-Diol G to three serum samples. The results (in ng/mL) are tabulated below:

Sample	Obs. Result	Exp. Result	Recovery %
1 Unspiked	1.7	-	-
+ 0.25(1:1,v/v)	0.9	0.98	91.8
+ 3.0(1:1,v/v)	2.6	2.35	102.4
+ 15.0(1:1,v/v)	25.4	25.85	98.3
2 Unspiked	8.8	-	-
+ 1.0(1:1,v/v)	4.7	4.9	95.9
+ 3.0(1:1,v/v)	6.0	5.9	105.3
+ 10(1:1,v/v)	9.9	9.4	101.7
3 Unspiked	2.9	-	-
+ 0.25(1:1,v/v)	1.4	1.5	93.3
+ 3.0(1:1,v/v)	2.9	2.95	98.3
+ 50(1:1,v/v)	29.3	26.3	111.4

LINEARITY

Three serum samples were diluted with calibrator A. The results (in ng/mL) are tabulated below:

Sample	Obs. Result	Exp. Result	Recovery %
1	21.8	-	-
1:2	10.2	10.9	93.6
1:4	5.5	5.45	99.1
1:8	2.7	2.73	98.9
2	25.2	-	-
1:2	13.4	12.6	106.3
1:4	7.4	6.3	117.5
1:8	3.5	3.2	109.4
3	27.4	-	-
1:2	14.8	13.7	108.0
1:4	7.4	6.85	108.0
1:8	3.5	3.43	103.0

EXPECTED NORMAL VALUES

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

Group	Range (ng/mL)
Males	1.53-14.82
Premenopausal	0.22-4.64
Postmenopausal	0.61-3.71
Puberty (Female)	0.51-4.03

REFERENCES

1. Jacobi GH, Wilson JD. Formation of 5α-androstane-3α, 17β-diol by Normal and Hypertrophic Human Prostate. J Clin Endocrinol Metab. 1977; 44(1):107–15.
2. Deslypere JP, et al. Plasma 5α-androstane-3α,17β-diol and Urinary 5α-androstane-3α, 17βdiol glucuronide, Parameters of Peripheral Androgen Action: A Comparative Study. J Clin Endocrinol Metab. 1982; 54(2):386–91.
3. Moghissi E, et al. Origin of Plasma Androstanediol Glucuronide in Men. J Clin Endocrinol Metab. 1984; 59(3):417–21.
4. Scanlon M J, et al. Serum Androstanediol Glucuronide Concentrations in Normal and Hirsute Women and Patients with Thyroid Dysfunction. Clin Endocinol (Oxf). 1988; 29(5):529–38.
5. Reiner B J, et al. Serum 3 α-androstanediol Glucuronide Measurements in Sexually Mature Women with Congenital Adrenal Hyperplasia During Therapy. J Clin Endocrinol Metab. 1989; 69(1)105–9.
6. Vexiau P, et al. Increase in Plasma 5α-androstane-3α,17β-diol Glucuronide as a Marker of Peripheral Androgen Action in Hirsutism: A Side-effect Induced by Cyclosporine A. J Steroid Biochem. 1990; 35(1):133–7.
7. Pang S, et al. 3α-androstanediol Glucuronide in Virilizing Congenital Adrenal Hyperplasia: A Useful Serum Metabolic Marker of Integrated Adrenal Androgen Secretion. J Clin Endocrinol Metab. 1991; 73(1):166–74.
8. Riddick LM, et al. 3α-androstanediol Glucuronide in Premature and Normal Pubarche. J Clin Endocrinol Metab. 1991; 72(1):46–50.
9. Check JH, et al. Falsely Elevated Steroidal Assay Levels Related to Heterophile Antibodies Against Various Animal Species. Gynecol Obstet Invest. 1995; 40(2):139–40.

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