DRG

DRG® 3α Diol G CLA (CLA-4649)

CE

RUO in the USA

Revised 20 Aug. 2010 rm (Vers. 1.1)

1 INTENDED USE

For the determination of 3α Diol G in human serum by chemiluminescence immunoassay (CLA).

In the United States, this kit is intended for Research Use Only.

2 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 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.
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, do not use pipettes in which this 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.

3 LIMITATIONS

1. All the reagents within the kit are calibrated for the direct 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 may be used to dilute any high serum samples. The use of any other reagent may lead to false results.

4 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.

5 CHEMICAL HAZARDS

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

6 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.

7 SPECIMEN PRETREATMENT

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

8 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. Plate shaker
5. Microwell plate luminometer

9 REAGENTS PROVIDED AND PREPARATION

1. Rabbit Anti-3α Diol G 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.

RUO in the USA

Revised 20 Aug. 2010 rm (Vers. 1.1)

2. 3α Diol G -Horseradish Peroxidase (HRP) Conjugate Concentrate - Requires Preparation.
Contents: 3α Diol G-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 1:50 in assay buffer before use (eg. 40 µL of HRP in 2 ml of assay buffer). If the whole plate is to be used dilute 240 µL of HRP in 12 ml of assay buffer. Discard any that is left over.

3. 3αDiol 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 vial labels for exact concentrations.

Calibrator	Concentration	Volume/Vial
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. Control - Ready To Use.
Contents: One vial containing 3α Diol G in a protein-based buffer with a non-mercury preservative. Prepared by spiking bufer 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 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 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.
*Warm to completely dissolve before use.

7. Cheminluminescence 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. Cheminluminescence 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. Cheminluminescence 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.

9.1 Preparation of Working Substrate Solution

Mix **1 part** of the chemiluminescence substrate **reagent A** with **1 part** of **reagent B** and dilute this mixture **1:6 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 12 ml of reagent C.
Total volume=14 ml of working substrate solution.

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

10 ASSAY PROCEDURE

Specimen Pretreatment: None.

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.

Procedure:

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 15 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 100 µL of chemiluminescence working substrate solution into each well.
   (We recommend using a multichannel pipette).
7. Incubate on a plate shaker (approximately 200 rpm) for 15 minutes at room temperature.
8. Measure the RLUs in each well on a microplate luminometer within 20 minutes after addition of the substrate.

11 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**

Calibrator	RLU 1 x 10^3	RLU 2 x 10^3	Mean RLU x 10^3	RLU/RLUMAX (%)
A, 0 ng/ml	1914	1917	1916	100
B, 0.25ng/ml	1669	1663	1671	87
C, 1 ng/ml	1181	1159	1170	61
D, 3 ng/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.

11.1 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

12 REFERENCES

1. Gunther, H.J. and Wilson, J.D., Formation of 5α-Androstane-3α,17βdiol by normal and hypertrophic human prostate. J. Clin. Endocrinol. Metab. 44/1:107 -115, 1977.
2. Deslypere, J.P., et al., Plasma 5α-Androstane-3α,17βdiol and urinary 5α-Androstane-3α,17βdiol glucuronide, parameters of peripheral androgen action: A comparative study. J. Clin. Endocrinol. Metab. 54/2:386-391, 1982.
3. Moghissi, E., et al., Origin of plasma androstanediol glucuronide in men. J. Clin. Endocrinol. Metab. 59/3:417- 421, 1984.
4. Scanlon, M.J. et al., Serum androstanediol glucuronide concentrations in normal and hirsute women and donors with thyroid dysfunction. Clin. Endocinol. 29:529-538, 1988.
5. Reiner, B.J., et al., Serum 3α-Androstanediol glucuronide measurements in sexually mature women with congenital adrenal hyperplasia during therapy. J. Clin. Endocrinol. Metab. 69-105-109, 1989.
6. Vexiau, P., et al., Increase in plasma 5α-Androstane-3α,17βdiol glucuronide as a marker of peripheral androgen action in hirsutism: A side-effect induced by cyclosporine A. J. Steroid Biochem. 35/1:133-137, 1990.
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. 73/1:166-174, 1991.
8. Riddick, L., et al., 3α-Androstanediol glucuronide in premature and normal pubarche. J. Clin. Endocrinol. Metab. 72:46-50, 1991.
9. Check, J.H., et al, Falsely elevated steroidal assay levels related to heterophile antibodies against various animal species. Gynecol Obstet Invest 40:139-1 40, 1995.