Monobind Inc.
Lake Forest, CA 92630, USA

AccuLite
CLIA Microwells

Androstenedione (ANST) Test System

Product Code: 12475-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Androstenedione Concentration in Human Serum or Plasma by a Microplate Enzyme Immunoassay, Chemiluminescence

2.0 SUMMARY AND EXPLANATION OF THE TEST

Androstenedione (ANST) is a steroid hormone (molecular weight of 290.4 daltons) that circulates in blood bound to sex hormone binding protein (SHBG). Its affinity to SHBG, is less than testosterone or dihydrotestosterone but greater than estrogens.

Androstenedione levels are found to have high diurnal variability. The highest concentrations are found in the morning. At puberty, androstenedione levels rise but fall after menopause. Higher levels are measured during pregnancy.

Androstenedione is secreted predominately by the adrenal glands partially regulated by adrenocorticotrophic hormone (ACTH). In addition to ACTH stimulation, ANST is produced in the testes and ovaries from adrenal secreted dehydroepiandrosterone sulfate (DHEAS). ANST is a critical sex steroid precursor.

High level in women can cause symptoms of hyperandrogenism. Increased concentrations are found in women with hirsutism (mostly in common with other androgens). Men are normally asymptomatic, but through peripheral conversion to estrogens can occasionally results in symptoms of mild estrogen excess. Most elevated levels of ANST are idiopathic. However, androgen producing tumors or gonadal tumors produces pronounced higher levels of ANST.

3.0 PRINCIPLE

Competitive Enzyme Immunoassay (TYPE 5):
The essential reagents required for a solid phase enzyme immunoassay include immobilized antibody, enzyme-antigen conjugate and native antigen.
Upon mixing immobilized antibody, enzyme-antigen conjugate and a serum containing the native antigen, a competition reaction results between the native antigen and the enzyme-antigen conjugate for a limited number of insolubilized binding sites.

The interaction is illustrated by the following equation:

$$^{Enz}Ag + Ag + Ab_{C.W.} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} AgAb_{C.W.} + {}^{Enz}AgAb_{C.W.}$$

$Ab_{C.W.}$ = Monospecific Immobilized Antibody (Constant Quantity)
Ag = Native Antigen (Variable Quantity)
^{Enz}Ag = Enzyme-antigen Conjugate (Constant Quantity)
$AgAb_{C.W.}$ = Antigen-Antibody Complex
$^{Enz}AgAb_{C.W.}$ = Enzyme-antigen Conjugate -Antibody Complex
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Disassociation
$K = k_a / k_{-a}$ = Equilibrium Constant

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is inversely proportional to the native antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS

Materials Provided

A. ANST Calibrators – 1ml/vial - Icons A-F
Six (6) vials of serum reference for androstenedione at concentrations of 0 (**A**), 0.1 (**B**), 0.3 (**C**), 1.0 (**D**), 30. (**E**), 10.0 (**F**) in ng/ml. Store at 2-8°C. A preservative has been added. The calibrators can be expressed in molar concentrations (nmol/L) by multiplying by 3.49.
For example: 1ng/ml x 3.49= 3.49 nmol/L

B. ANST Tracer Reagent – 12.0 ml/vial – Icon
One (1) vial contains ANST (Analog)-horseradish peroxides (HRP) conjugate in a protein-stabilizing matrix red with dye. Store at 2-8°C.

C. ANST Light Reaction Wells – 96 wells – Icon
One 96-well white microplate coated with ANST-specific rabbit IgG and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

D. Wash Solution Concentrate – 20ml/vial - Icon
One (1) vial contains a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

E. Signal Reagent A – 7.0ml/vial - Icon C[A]
One (1) vial contains luminol in a buffer. Store at 2-8°C.

F. Signal Reagent B – 7.0ml/vial - Icon C[B]
One (1) vial contains hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C.

G. Product Instructions.

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on the label.**
Note 3: Above reagents are for a single 96-well microplate**.**

4.1 Required But Not Provided:
1. Pipette capable of delivering 0.025 and 0.100ml (25 and 100µl) with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.050ml (50µl), 0.100ml (100µl) and 0.350ml (350µl) volumes with a precision of better than 1.5%.
3. Test tubes for Signal Reagent (See Reagent Preparation)
4. Microplate washer or a squeeze bottle (optional).
5. Microplate Luminometer
6. Absorbent Paper for blotting the microplate wells.
7. Plastic wrap or microplate cover for incubation steps.
8. Vacuum aspirator (optional) for wash steps.
9. Timer.
10. Quality control materials.

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA required tests. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe Disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum or heparanised plasma in type, and taken with the usual precautions in the collection of venipuncture samples. For accurate comparison to establish normal values, a fasting morning serum sample should be obtained. The blood should be collected in a redtop (with or without gel additives) venipuncture tube(s) or for plasma use evacuated tube(s) containing heparin. Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum or plasma from the cells.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.050ml (50µl) of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and high range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. **Wash Buffer**
Dilute contents of wash solution concentrate to 1000ml with distilled or deionized water in a suitable storage container. Diluted buffer can be stored at 2-30°C for up to 60 days.
2. **Working Signal Reagent Solution -** Store at 2 - 8°C.
Determine the amount of reagent needed and prepare by mixing equal portions of Signal Reagent A and Signal Reagent B in a clean container. For example, add 1 ml of A and 1ml of B per two (2) eight well strips (A slight excess of solution is made). **Discard the unused portion if not used within 36 hours after mixing.** If complete utilization of the reagents is anticipated, within the above time constraint, pour the contents of Signal Reagent B into Signal Reagent A and label accordingly.

Note: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20-27°C).
*****Test Procedure should be performed by a skilled individual or trained professional*****

1. Format the microplates' wells for each serum reference, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
2. Pipette 0.025 ml (25 µL) of the appropriate serum reference, control or specimen into the assigned well.
3. Add 0.100 ml (100µl) of the ANST Tracer Reagent to all wells.
4. Swirl the microplate gently for 20-30 seconds to mix.
5. Cover and incubate for 45 minutes at room temperature.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
7. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat four (4) additional times for a total of five (5) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat four (4) additional times.**
8. Add 0.100 ml (100µl) of working signal reagent solution to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time differences between wells.**
DO NOT SHAKE THE PLATE AFTER SIGNAL ADDITION
9. Incubate at room temperature for five (5) minutes in the dark.
10. Read the relative light units in each well with a chemiluminescence microplate reader for 0.5-1.0 seconds. **The results should be read within 30 minutes after adding the working Signal Reagent.**

Note: Dilute the samples suspected of concentrations higher than 10 ng/ml 1:5 and 1:10 with ANST '0' ng/ml calibrator.

10.0 CALCULATION OF RESULTS

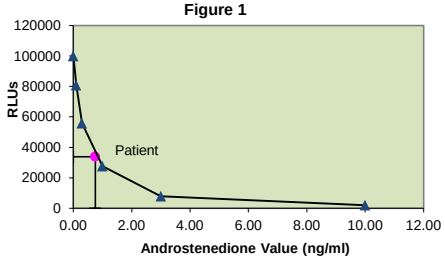
A dose response curve is used to ascertain the concentration of ANST in unknown specimens.
1. Record the RLU obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the RLU for each duplicate serum reference versus the corresponding ANST concentration in ng/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Connect the points with a best-fit curve.
4. To determine the concentration of ANST for an unknown, locate the average RLU of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in ng/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average RLU (33888) intersects the dose response curve at 0.75ng/ml ANST concentration (See Figure 1).

Note: Computer data reduction software designed for CLIA assay may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

EXAMPLE 1

Sample I.D.	Well Number	RLU (A)	Mean RLU (B)	Value (ng/ml)
Cal A	A1	100569	100000	0
	B1	99431		
Cal B	C1	82322	80884	0.1
	D1	79447		
Cal C	E1	56228	56839	0.3
	F1	57430		
Cal D	G1	26909	27743	1.0
	H1	28578		
Cal E	A2	7905	7929	3.0
	B2	7953		
Cal F	C2	2108	2053	10.0
	D2	1999		
Pat# 1	E2	32689	33888	0.75
	F2	35087		

Figure 1



***** The data presented in Example 1 and Figure 1 is for illustration only and **should not** be used in lieu of a dose response curve prepared with each assay. In addition, the RLUs of the calibrators have been normalized to 100,000 RLUs for the A calibrator (greatest light output). This conversion minimizes differences caused by efficiency of the various instruments that can be used to measure light output.

11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The Dose Response Curve should be within established parameters.
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product is available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of signal reagent initiates a kinetic reaction, therefore the signal reagent(s) should be added in the same sequence to eliminate any time-deviation during reaction.
6. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
7. Use components from the same lot. No intermixing of reagents from different batches.
8. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind IFU may yield inaccurate results.
9. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
10. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
11. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional***.
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for the procedure have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays. *(Boscato LM Stuart MC.'Heterophilic antibodies: a problem for all immunoassays' Clin. Chem 1988:3427-33).* For diagnostic purposes the results from this assay should be used in combination with clinical examination, patient's history, and, all other clinical findings.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
6. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

13.0 EXPECTED RANGES OF VALUES

In agreement with established reference intervals for a "normal" adult population and females during gestation, the expected ranges for the Androstenedione AccuLite® CLIA Test System are detailed in Table 1.

TABLE 1
Expected Values for the Androstenedione Test System

Adults	
Females	0.3-2.0 ng/ml
Males	0.4-1.5 ng/ml

It is important to keep in mind that establishment of a range of values, which can be expected to be found by a given method for a population of "normal" persons is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons, each laboratory should depend upon the range of expected values established by the manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precisions of the ANST AccuLite® CLIA test system were determined by analyses on three different levels of pool control sera. The number (N), mean value (X), standard deviation (σ) and coefficient of variation (C.V.) for each of these control sera are presented in Table 2 and Table 3.

TABLE 2
Within Assay Precision (Values in ng/ml)

Sample	N	X	σ	C.V.
Low	12	0.61	0.05	8.2%
Normal	12	2.18	0.07	3.2%
High	12	5.88	0.25	4.3 %

TABLE 3
Between Assay Precision (Values in ng/ml)

Sample	N	X	σ	C.V.
Low	10	0.65	0.06	9.2%
Normal	10	2.32	0.10	4.3%
High	10	5.68	0.33	5.8%

*As measured in ten experiments in duplicate over a ten day period.

14.2 Sensitivity

The ANST AccuLite® CLIA Test System has a sensitivity of 0.025ng/ml.

14.3 Accuracy

14.3.1 Linearity

The linearity of the ANST AccuLite® CLIA Test System was tested by diluting several serum samples containing high levels of Androstenedione with the "0 ng/ml" serum reference. The system was determined to have excellent linearity with between 90-110% recovery for three samples diluted 1:2, 1:4, 1:8 and 1:16.

14.3.2 Recovery

The recovery of the ANST AccuLite® CLIA Test System was calculated for three patient samples spiked with 0.5, 1.0, and 2.0 and 5.0ng/ml Androstenedione. Recoveries were determined to be within 10% of the expected values for all samples.

14.4 Specificity

The % cross reactivity of the androstenedione antibody to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of androstenedione needed to displace the same amount of labeled analog.

Substance	Cross Reactivity
Androstenedione	100.000
Testosterone	0.235
5α-Dihydrotestosterone	0.033
Progesterone	0.047
DHEA sulfate	0.005
Cortisol	<0.001
Aldosterone	<0.001
Estradiol (17αorβ)	<0.001
Estriol	<0.001
DHEA sulfate	0.005
Pregnenolone	<0.001

15.0 REFERENCES

1. Sciarra F, Tostu V: Androgen-secreting adrenal tumors, *Minera Endocrinol,* 1995;20:63-66
2. Allolio B. Arit W,DHEA treatment myth or reality, *Trends Endocrine Metab,* 2002;13:288-294
3. Collett-Solberg P: Congenital adrenal hyperplasia from genetics and biochemistry to clinical practice, part 1.*Clin Pediatr,* 2001:40;1-16
4. Kicman AT, Bassindale T, Cowan, DA, Dale S, Hutt AJ and Leeds, Effect of androstenedione ingestion on plasma testosterone in young women, a dietary supplement with potential health risks, *Clin Chem,*2003, 49:167-169.

Effective Date: 2023-FEB-27 Rev. 1 **DCO: 1611**
MP12475 **Product Code: 12475-300**

Size		96(A)	192(B)
Reagent (fill)	A)	1ml set	1ml set
	B)	1 (12ml)	2 (12ml)
	C)	1 plate	2 plates
	D)	1 (20ml)	1 (20ml)
	E)	1 (7ml)	2 (7ml)
	F)	1 (7ml)	2 (7ml)

For Orders and Inquires, please contact

Monobind Inc.
100 North Pointe Drive
Lake Forest, CA 92630 USA

Tel: +1 949.951.2665 Mail: info@monobind.com
Fax: +1 949.951.3539 Fax: www.monobind.com

IVD 2° 8° CE
EC REP **CEpartner4U,** Esdoornlaan 13
3951 DBMaarn, The Neatherlands
www.cepartner4u.eu

Please visit our website to learn more about our products and services.

Glossary of Symbols
(EN 980/ISO 15223)