EIASON® Aldosterone

IVD

Enzyme immunoassay for the quantitative determination of Aldosterone
in human serum, plasma (EDTA-, heparin- or citrate plasma) or urine

Kit instruction

For in-vitro use only

Product of

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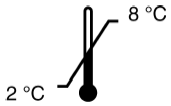
REF E03-053-96Q

2 °C 8 °C

CE

Used IFU-Symbols

Symbol	English	Symbol	English
IVD	In vitro diagnostic device	SUB	Substrate
REF	Order number	CAL 0-5	Calibrators
	Product of	STOP	Stop solution
	Storage	WASH	Wash Buffer
CE	European Conformity	CONJ	Enzym conjugate
	Expiry date	CO1 CO2	Control low Control high
LOT	Batch code	MPL	Microplate

Intended Use

The EIASON® Aldosterone ELISA is an enzyme immunoassay for the quantitative in vitro diagnostic measurement of Aldosterone in human serum, plasma (EDTA-, heparin- or citrate plasma) and urine

Principle of the test

The RIASON® Aldosterone ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the principle of competitive binding.
The microtiter wells are coated with a polyclonal rabbit antibody directed towards an antigenic site of the aldosterone molecule. Endogenous aldosterone of a patient sample competes with an aldosterone-horseradish peroxidase conjugate for binding to the coated antibody. After incubation the unbound conjugate is washed off.
After addition of the substrate solution, the intensity of colour developed is inversely proportional to the concentration of aldosterone in the patient sample.

Summary

The steroid hormone aldosterone is a potent mineral corticoid that is produced by the zona glomerulosa of the adrenal cortex in the adrenal gland. The synthesis and release are controlled by the renin-angiotensin-aldosterone system (RAAS)[1], as well as by plasma potassium concentration [2], the pituitary peptide ACTH, and by the blood pressure via pressure sensitive baroreceptors in the vessel walls of nearly all large arteries of the body[3]. Aldosterone binds to mineralocorticoid receptors (MR) and triggers the transcription of hormone-responsive genes. In consequence, aldosterone increases the blood pressure by reabsorption of sodium and water from the distal tubules of the kidney into the blood, secretion of potassium into the urine, and elevation of circulating blood volume. Chronic overproduction and secretion of aldosterone leads to hypertension. Aldosterone activity is reduced in Addison's disease and increased in Conn's syndrome.
Measurement of aldosterone levels in serum in conjunction with plasma renin levels (aldosterone/renin-ratio; ARR) can be used to differentiate between primary and secondary aldosteronism [4,8,9].

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 [5]:

- Primary aldosteronism caused by an adenoma of one or both adrenals.
- 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 [6]. In addition, pharmacological modulation of nuclear hormone receptors is a common strategy for the treatment of cardiovascular disease [7]. Therefore, determining the effects of such treatments on the RAAS is of increasing value in evaluating the safety and efficacy of new therapeutics.In summary, the precise and accurate measurement of serum aldosterone by enzyme immunoassay can be an important adjunct to a diagnostic laboratory battery for the differential diagnosis of hypertensive disease.

Warnings and precautions

The EIASON® Aldosterone ELISA Kit is for in vitro diagnostic use only and is not for internal use in humans or animals. This product must be used strictly in accordance with the instructions set out in the Package Insert. IASON will not be held responsible for any loss or damage (except as required by statute) caused, arising out of non-compliance with the instructions provided.
CAUTION: this kit contains material of human and/or animal origin. Handle kit reagents as if capable of transmitting an infectious agent.
Source material from Human origin which is used in this kit was tested and found negative for HbsAG and HIV as well as for HCV antibodies. However, since there is

no diagnostic procedure that excludes these agents with 100 percent certainty all components should be handled as potentially hazardous material.
Appropriate precautions and good laboratory practices must be used in the storage, handling and disposal of the kit reagents. Disposal of kit reagents should be in accordance with local regulations.

Shelf life and storage of reagents

When stored at 2 °C to 8 °C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.
Opened reagents must be stored at 2 °C to 8 °C. Microtiter wells must be stored at 2°C to 8°C. Once the foil bag has been opened, care should be taken to close it tightly again.
Opened kits retain activity for two months if stored as described above.

Specimen collection and preparation

Serum/ plasma samples

Serum or plasma (EDTA-, heparin- or citrate plasma) and urine can be used in this assay. Do not use haemolytic, icteric or lipaemic specimens.
Please note: Samples containing sodium azide should not be used in the assay.

Serum samples collection:

Collect blood by venipuncture (e.g. Sarstedt Monovette for serum), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Patients receiving anticoagulant therapy may require increased clotting time.

Plasma samples collection:

Whole blood should be collected into centrifuge tubes containing anti-coagulant (e.g. Sarstedt Monovette with the appropriate plasma preparation) and centrifuged immediately after collection.

Specimen storage

Specimens should be capped and may be stored for up to 4 days at 2 °C to 8 °C prior to assaying. Specimens held for a longer time (up to two months) should be frozen only once at -20 °C prior to assay. Thawed samples should be inverted several times prior to testing.

Specimen dilution

If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be diluted with CAL0 and reassayed.
For the calculation of the concentrations this dilution factor has to be taken into account.
Example:
a) dilution 1:10: 10 µL sample + 90 µL CAL0 (mix thoroughly)
b) dilution 1:100: 10 µL dilution a) 1:10 + 90 µL CAL0 (mix thoroughly).

Urine samples

Aldosterone concentration can also be determined from urine samples. However, urine samples must be pre-treated before analysis. This will need additional reagents that are not included in this kit, but can be ordered separately (REF E03-053-96Q - URIN).

Urine samples collection:

First clean genital area with mild desinfectant to prevent contamination. Then collect clean-catch midstream urine in an appropriate sterile container. Directly after collection, the urine should be centrifuged for 5 - 10 minutes (e.g. at 2,000 g) to remove cellular debris. Use supernatant for analyte quantification. The supernatant may be stored for up to 8 hours at 2 °C to 8 °C prior to assaying. Specimens held for a longer time should be frozen at -20 °C. Thawed supernatant should be inverted several times prior to testing.

Urine pre-treatment (the needed reagents are available at IASON: REF E03-053-96Q-URIN)

1. Secure the desired number of vials (e.g. 0.5 - 1.5 mL plastic tubes; not included in this kit).
2. Dispense 25 µL of urine with new disposable tips into appropriate tubes.
3. Dispense 25 µL Release Reagent into each tube. Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
4. Incubate over night at 2 °C to 8 °C.
5. Add 25 µL Neutralization Reagent to each tube and mix thoroughly.
6. Add 400 µL Dilution Buffer to each tube and mix thoroughly
   (This pre-treatment leads to a 1:19 dilution. Therefore the dilution factor 19 has to be taken into account for calculation of the final concentration of the urine sample.)
7. Transfer 50 µL of pre-treated and diluted urine samples directly to the microtiter well and continue with step 3 of *Assay procedure*.

Specimen dilution

If in an initial assay, an urine sample is found to contain more than the highest standard, the pre-treated and diluted urine sample can be further diluted with Dilution Buffer and reassayed as described in Assay procedure.
For the calculation of the concentrations this dilution factor has to be taken into account too.
Example:
a) dilution 1:10: 10 µL pre-treated and diluted urine sample + 90 µL Dilution Buffer (mix thoroughly) (final dilution factor = 19 x 10 = 190)

Materials provided

Allow all reagents to reach room temperature before use

1. **MPL** Microtiterwells, 12 x 8 (break apart) strips, 96 wells coated with anti-aldosterone antibody (polyclonal rabbit).

2. **CONJ** 1 vial, 20 mL, **ready to use**, aldosterone conjugated to horseradish peroxidase; contains non-mercury preservative.

3. **CAL 0-5** 6 vials (lyophilized); Reconstitute the lyophilized contents of the standard vials with 1.0 mL deionized water and let stand for at least 10 minutes. Mix several times before use.
   Concentrations: 0 – 20 – 80 – 200 – 500 – 1000 pg/mL
   Conversion: 1 pg/mL corresponds to 2.77 pmol/L
   Note: The reconstituted standards are stable for 8 weeks at 2 °C to 8 °C. For longer storage freeze - only once - at -20 °C.

4. **CO 1-2** Control Low & High, 2 vials (lyophilized), Reconstitute the lyophilized content of the controls with 1.0 mL deionized water and let stand for at least 10 minutes.Mix several times before use
   For control values and ranges please refer to vial label or QC-Datasheet. Note: The reconstituted controls are stable for 8 weeks at 2 °C to 8 °C. For longer storage freeze - only once - at -20 °C.

5. **WASH** Wash Solution, 1 vial, 30 mL (40X concentrated), dilute 30 mL of concentrated WASH with 1170 mL deionized water to a final volume of 1200 mL. The diluted WASH is stable for 2 weeks at room temperature.

6. **SUB** Substrate Solution, 1 vial, 25 mL, **ready to use**, Tetramethylbenzidine (TMB).

7. **STOP** **StopSolution** 1 vial, 14 mL, **ready to use**, contains 0.5 M H2SO4,
   Avoid contact with the stop solution. It may cause skin irritations and burns.

Materials required but not provided in the kit

- A microtiter plate calibrated reader (450 ± 10 nm)
- Calibrated variable precision micropipettes.
- Absorbent paper.
- Distilled or deionized water
- Timer
- Scale paper or semi-logarithmic graph paper or software for data reduction
- Optional: Reagents for determination of Aldosterone in urine (REF E03-053-96Q-URIN) – Contents:
  1) Release Reagent, 1 vial, 3 mL, ready to use. Containing 1M HCl. Avoid contact with Release Reagent. It may cause skin irritation.
  2) Neutralization Buffer, 1 vial, 3 mL, ready to use. Containing Tris buffer, pH 8,5.
  3) Dilution Buffer, 2 vials, 25 mL each, ready to use. Containing PBS.
- Optional: Plastic tubes (e.g. 0.5 - 1.5 mL) for pre-treatment of urine samples.

Assay procedure

General remarks

All reagents and specimens must be allowed to come to room temperature (21 °C to 26 °C) before use. All reagents must be mixed without foaming.
Once the test has been started, all steps should be completed without interruption.
Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination.
Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
As a general rule the enzymatic reaction is linearly proportional to time and temperature.
Each run must include a standard curve.

Test procedure

1. Secure the desired number of Microtiter wells in the frame holder.
2. Dispense 50 µL of each CAL 0-5, *CO 1-2* and samples with new disposable tips into appropriate wells. For urine samples dispense 50 µL of the pre-treated and diluted urine samples (as in Urine pre-treatment step7 described)
3. Inkubate for 30 minutes at room temperature.
4. Dispense 150 µL CONJ into each well. Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
5. Incubate for 60 minutes at room temperature.
6. Briskly shake out the contents of the wells. Rinse the wells 5 x with 400 µL diluted WASH per well (if a plate washer is used) – or 5 x with 300 µL/well for manual washing.

   Strike the wells sharply on absorbent paper to remove residual droplets.

   Important note: The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
7. Add 200 µL of SUBS to each well.
8. Incubate for 30 minutes at room temperature.
9. Stop the enzymatic reaction by adding 100 µL of STOP to each well.
10. Determine the absorbance (OD) of each well at 450 ± 10 nm with a microtiter plate reader. It is recommended that the wells be read within 10 minutes after adding the STOP.

or fully automated on:

- **IASON® Quardette**
- **IASON® PersonalLab**
- **IASON® Gladiator**

Calculation

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Using scale paper or semi-logarithmic graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. The concentration of the serum / plasma samples can be read directly from this standard curve. For urine samples the concentration read from the standard curve, has to be multiplied with the dilution factor of 19.
6. Samples with concentrations higher than that of the highest standard have to be further diluted or reported as > 1000 pg/mL. For the calculation of the concentrations this dilution factor has to be taken into account too.

Final calculation for urine samples

Calculate the 24 hours excretion for each urine sample: µg/24 h = µg/L x L/24 h

Example:

Concentration for urine sample read from the standard curve = 500 pg/mL

Result after correction with the dilution factor 19 = 9500 pg/mL

9500 pg/mL / 1000 = 9.5 µg/L

Total volume of 24 h-urine = 1.3 L (example)

9.5 µg/L × 1.3 L/24 h = 12.35 µg/24 h

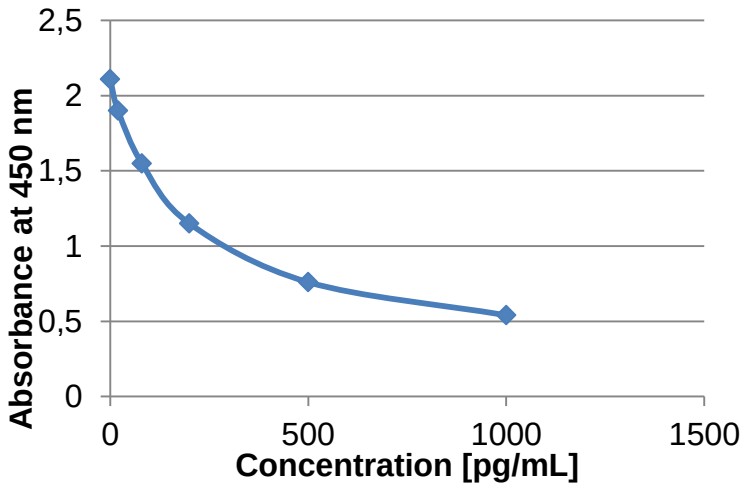
Typical data

The following data is for demonstration only and **cannot** be used in place of data generations at the time of assay.

Calibrator	Concentration [pg/ml]	Optical units at 450 nm
CAL0	0	2.13
CAL1	20	1.92
CAL2	80	1.60
CAL3	200	1.15
CAL4	500	0.78
CAL5	1000	0.56

Typical calibration curve

Sample curve only. **Do not** use to calculate results.



Expected normal values

It is strongly recommended that each laboratory should determine its own normal and abnormal values.
In a study conducted with serum samples of apparently normal healthy adults, using the EIASON® Aldosterone the following values are observed:

Healthy adults	Valid N	Mean [pg/mL]	Median [pg/mL]	5. percentile [pg/mL]	95. percentile [pg/mL]
Upright position	60	68.8	52.5	13.3	231.4
Supine position	60	62.8	50.9	12.0	157.5

These results correspond well to published reference ranges [8,9].

In a study conducted with apparently normal healthy adults, using the EIASON® Aldosterone (E03-053-96Q) and the EIASON® Renin (E03-005-96) the following Aldosterone-Renin ratios were determined in plasma:

Ratio Aldosterone-Renin [pg/mL / pg/mL]

n	Mean	Median	99th percentile	95th percentile	5th percentile	1st percentile
89	8.68	5.30	49.65	28.06	0.68	0.45

In a study conducted with **urine samples** of apparently normal healthy adults, using the EIASON® Aldosterone the following values are observed:

n	Mean [µg/24h]	Median [µg/24h]	5th percentile	95th percentile
40	11.34	9.40	3.55	23.01

These results correspond well to published reference ranges [8.]

Quality control

Good laboratory practice requires that controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance.
It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels.
The controls and the corresponding results of the QC-Laboratory are stated in the QC certificate added to the kit. The values and ranges stated on the QC sheet always refer to the current kit lot and should be used for direct comparison of the results.
It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.
Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials patient results should be considered invalid.
In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods. After checking the above mentioned items without finding any error contact your distributor or IASON directly.

PERFORMANCE CHARACTERISTICS

Assay dynamic range

The range of the assay is between 5.7 pg/mL – 1000 pg/mL.

Specificity of antibodies (cross-reactivity)

The following compounds were tested for cross-reactivity of the assay:

Steroid	% Cross Reactivity
3β,5α Tetrahydroaldosterone	17.2 %
3β,5β Tetrahydroaldosterone	0.12 %
Prednisolone	0.017 %
Cortisol	< 0.003 %
11-Deoxycortisol%	< 0.003 %
Progesterone	< 0.003 %
Testosterone	< 0.002 %
Androstenedione	< 0.002 %

Sensítivity

The analytical sensitivity of the EIASON® Aldosterone was calculated by subtracting 2 standard deviations from the mean of 20 replicate analyses of the CAL0 and was found to be < 5.7 pg/mL.

Reproducibility

Intra-assay precision			Inter-assay precision		
Sample	**Mean [pg/mL]**	**CV%**	**Sample**	**Mean [pg/mL]**	**CV%**
Serum 1 (n=20)	85.1	9.7	Serum 1 (n=40)	101.0	9.9
Serum 2 (n=20)	210.3	7.4	Serum 2 (n=40)	315.1	8.6
Serum 3 (n=20)	532.2	3.9	Serum 3 (n=40)	656.8	9.4
Urine 1 (n=20)	191.8	5.0	Urine 1 (n=32)	386.7	11.5
Urine 2 (n=20)	391.3	5.6	Urine 2 (n=32)	444.0	11.1
Urine 3 (n=20)	936.8	3.8	Urine 3 (n=32)	876.7	10.4

Recovery

Samples have been spiked by adding aldosterone solutions with known concentrations in a 1:1 ratio. The % recovery has been calculated by multiplication of the ratio of the measurements and the expected values with 100 (expected value = (endogenous aldosterone + added aldosterone) / 2; because of a 1:2 dilution of serum with spike material).

		Serum 1	Serum 2	Serum 3
Concentration [pg/mL]		82.7	96.1	167.9
Average Recovery		112.5	111.0	106.8
Range of Recovery [%]	from	108.2	108.9	92.4
	to	114.6	114.5	114.8

Linearity

		Serum 1	Serum 2	Serum 3	Urine 1	Urine 2	Urine 3
Concentration [pg/mL]		600.5	546.2	672.0	559.0	645.0	464.0
Average Recovery		98.4	95.5	96.4	106.8	98.2	98.0
Range of Recovery [%]	from	95.5	87.8	86.0	104.5	87.8	86.2
	to	103.0	103.6	102.5	111.6	107.9	105.6

Limitation of use

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the package insert instruction and with adherence to good laboratory practice.
Any improper handling of samples or modification of this test might influence the results.
Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.

Interfering substances

Haemoglobin (up to 4 mg/mL), Bilirubin (up to 0.125 mg/mL) and Triglyceride (up to 30 mg/mL) have no influence on the assay results.

Drug interferences

Until today no substances (drugs) are known to us, which have an influence to the measurement of aldosterone in a sample.

High-Dose-Hook Effect

No hook effect was observed in this test.

Legal aspects

Reliability of results

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.
The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. In case of any doubt or concern please contact IASON.

Therapeutic consequences

Therapeutic consequences should never be based on laboratory results alone even if all test results are in agreement with the items as stated under Reliability of Results. Any laboratory result is only a part of the total clinical picture of a patient.
Only in cases where the laboratory results are in acceptable agreement with the overall clinical picture of the patient should therapeutic consequences be derived.
The test result itself should never be the sole determinant for deriving any therapeutic consequences.

Liability

Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.
Claims submitted due to customer misinterpretation of laboratory results subject to point Therapeutic Consequences are also invalid. Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

Useful publications

1. Brown RD, Strott CA, and Liddle GW. Site of stimulation of Aldosterone biosynthesis by angiotensin and potassium.
1. J Clin Invest. (1972), 51 (6), 1413–8.
2. Bauer JH, Gauntner WC. Effect of potassium chloride on plasma renin activity and plasma aldosterone during sodium restriction in normal man. Kidney Int. (1979), 15 (3): 286–93.
3. Williams GH, Dluhy RG. Aldosterone biosynthesis. Interrelationship of regulatory factors. Am J Med. (1972), 53 (5), 595–605.
4. Tiu SC et al. The use of aldosterone-renin ratio as a diagnostic test for primary hyperaldosteronism and its test characteristics under different conditions of blood sampling. J Clin Endocrinol Metab. (2005), 90 (1), 72-8.
5. Mulatero P et al. Confirmatory tests in the diagnosis of primary aldosteronism.
6. Horm Metab Res. (2010), 42 (6), 406-10.
7. Quillo AR. Primary aldosteronism: results of adrenalectomy for nonsingle adenoma. J Am Coll Surg. (2011), 213 (1), 106-12.
8. Grossmann C and Gekle M. New aspects of rapid aldosterone signaling. Mol Cell Endocrinology (2009), 308 (1-2), 53-62.
9. Thomas L (editor). Renin-Angiotensin-Aldosteron-System (RAAS). Labor und Diagnose (2005); 1406-24.
10. Perschel FH et al. Rapid Screening test for primary hyperaldosteronism: ratio of plasma aldosterone to renin concentration determined by fully automated chemiluminescence immunoassays. Clin. Chemistry (2004); 50 (9), 1650-55.

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Pipetting scheme

All reagents must reach room temperature before use.

1. Pipetting	**CAL0-5** **50µL**	**CO1-2** **50µL**	**Samples** **50µL**
2. Inkubation	30 min at room temperature		
3. Pipetting	**CONJ** Mix well for 10 s		**150µL**
4. Incubation	1 hour at room temperature		
5. Washing	aspirate or decant wash 5x : add **400µL** of diluted **WASH** (**300µL** for manual washing) aspirate or decant and dry on an absorbent material		
6. Pipetting	**SUB**		**200µL**
7. Incubation	30 min at room temperature		
8. Pipetting	**STOP**		**100µL**
9. Reading	450nm (RF 620nm) reading within 10 min. Calculation: 4-parameter		

EXPECTED NORMAL VALUES – SERUM

Healthy adults	**5th-95th percentile range [pg/mL]**
Upright position	13.3 – 231.4
Supine position	12.0 – 157.5

EXPECTED NORMAL VALUES - URINE

	5th-95th percentile range [pg/mL]
Healthy adults	3.55 – 23.01