

Parameter™

Aldosterone Assay

Catalog Number KGE016

For the quantitative determination of Aldosterone concentrations in cell culture supernates, serum, plasma, and urine.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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MANUFACTURED AND DISTRIBUTED BY:

USA & Canada | R&D Systems, Inc.

614 McKinley Place NE, Minneapolis, MN 55413, USA
TEL: (800) 343-7475 (612) 379-2956 FAX: (612) 656-4400
E-MAIL: info@RnDSystems.com

DISTRIBUTED BY:

UK & Europe | R&D Systems Europe, Ltd.

19 Barton Lane, Abingdon Science Park, Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449 FAX: +44 (0)1235 533420
E-MAIL: info@RnDSystems.co.uk

China | R&D Systems China Co., Ltd.

24A1 Hua Min Empire Plaza, 726 West Yan An Road, Shanghai PRC 200050
TEL: +86 (21) 52380373 FAX: +86 (21) 52371001
E-MAIL: info@RnDSystemsChina.com.cn

INTRODUCTION

Aldosterone is a lipophilic cholesterol-derived steroid hormone that regulates blood pressure homeostasis (1). Due to the importance of Aldosterone in blood pressure homeostasis via the regulation of sodium and potassium balance, pharmacological modulation of Aldosterone synthesis is a highly active area of translational and clinical research (2). While Aldosterone is synthesized mainly in the adrenal zona glomerulosa, the steroidogenic enzymes necessary to produce Aldosterone *de novo* or from steroid precursors are also expressed in peripheral tissues including some cardiovascular and central nervous system tissues (3). As Aldosterone is able to pass freely through cell membranes, its concentration in the circulation and in local tissues is directly correlated to synthesis. The Renin-Angiotensin system, extracellular potassium concentrations, and Adrenocorticotropin Hormone (ACTH) are the primary regulators of Aldosterone biosynthesis. However, several additional biological factors can modulate its synthesis including Epinephrine, Vasoactive Intestinal Polypeptide, Serotonin, Ouabain, Atrial Natriuretic Peptide, Dopamine, Heparin, and Adrenomedullin (3, 4).

In the circulation, Aldosterone can be bound by Albumin and Corticosteroid-Binding Globulin (CBG) (5). Additional Aldosterone binding proteins in the plasma have been described but not fully characterized (6). Aldosterone binds and activates the Mineralocorticoid Receptor (MR), a member of the ligand-activated transcription factor superfamily (7). It also binds the Glucocorticoid Receptor, although with low affinity compared to Cortisol (8). Aldosterone additionally binds cell surface-localized receptors that activate non-genomic/MR-independent signaling (9-11). Although the identity of the membrane-localized Aldosterone-binding protein has not been established, Aldosterone has been shown to mediate rapid MR-independent effects *via* GPR30, a steroid-binding G protein coupled receptor, in the vasculature (12).

As Aldosterone is a primary regulator of blood pressure homeostasis, pathophysiological changes in Aldosterone plasma levels are associated with cardiovascular and renal disease. Low plasma concentrations of Aldosterone occur in individuals with genetic mutations in steroidogenic enzymes and those with Addison's disease (13, 14). Alternatively, elevated levels of plasma Aldosterone are associated with primary aldosteronism (PA) syndromes. PA can occur as a result of several underlying causes including mutations in the MR, adrenal hyperplasia, and several types of tumors (15, 16). Elevated plasma Aldosterone levels are also associated with hypertension and diabetes mellitus and can cause cardiovascular and renal tissue damage resulting in elevated mortality risk (17-21). Aldosterone is excreted in the urine where concentrations are elevated in individuals with PA and hypertension (22, 23). Plasma concentrations of Aldosterone are influenced by circadian rhythms and fluctuate throughout the day (24-26).

The Parameter Aldosterone assay is a 3.5 hour competitive enzyme immunoassay designed to measure Aldosterone in cell culture supernates, serum, plasma, and urine.

PRINCIPLE OF THE ASSAY

This assay is based on the competitive binding technique. A monoclonal antibody specific for Aldosterone becomes bound to the goat anti-mouse antibody coated onto the microplate. Following a wash to remove excess monoclonal antibody, Aldosterone present in a sample competes with a fixed amount of horseradish peroxidase (HRP)-labeled Aldosterone for sites on the monoclonal antibody. This is followed by another wash to remove excess conjugate and unbound sample. A substrate solution is added to the wells to determine the bound enzyme activity. The color development is stopped and the absorbance is read at 450 nm. The intensity of the color is inversely proportional to the concentration of Aldosterone in the sample.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, further dilute the samples with Calibrator Diluent and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, incubation time or temperature, and kit age can cause assay variability.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in this assay, the possibility of interference cannot be excluded.
- **Samples containing mouse or rat IgG may interfere with this assay.**

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- Pre-rinse the pipette tip when pipetting.
- Pipette standards and samples to the bottom of the wells. Add all other reagents to the side of the wells to avoid contamination.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Goat anti-mouse IgG Microplate	892575	96 well polystyrene microplate (12 strips of 8 wells) coated with a goat anti-mouse IgG antibody.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
Aldosterone Conjugate	894717	6 mL of Aldosterone conjugated to horseradish peroxidase with red dye and preservatives.	May be stored for up to 1 month at 2-8 °C.*
Aldosterone Standard	894718	Synthetic Aldosterone in a buffered protein base with preservatives; lyophilized. <i>Refer to the vial label for reconstitution volume.</i>	
Aldosterone Primary Antibody Solution	894719	11 mL of mouse monoclonal antibody to Aldosterone in a buffered protein base with blue dye and preservatives.	
Assay Diluent RD1-120	896033	6 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-69	896032	21 mL of a buffered protein base with preservatives.	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. <i>May turn yellow over time.</i>	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895926	11 mL of 2 N sulfuric acid.	
Plate Sealers	N/A	4 adhesive strips.	

* Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 500 mL graduated cylinder.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm (R&D Systems, Catalog # T4625-Q or T4625-1CEQ) or equivalent.
- Test tubes for dilution of standards and samples.
- Aldosterone Controls (optional; available from R&D Systems).

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain ProClin® which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Please refer to the MSDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernates - Remove particulates by centrifugation. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Citrate plasma has not been validated for use in this assay.
Grossly hemolyzed samples are not suitable for use in this assay.*

Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter, and assay immediately or aliquot and store at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Cell culture supernate, serum, and plasma samples require a 3-fold dilution. A suggested 3-fold dilution is 100 μ L of sample + 200 μ L of Calibrator Diluent RD5-69.

Urine samples require a 10-fold dilution. A suggested 10-fold dilution is 30 μ L of sample + 270 μ L of Calibrator Diluent RD5-69.

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REAGENT PREPARATION

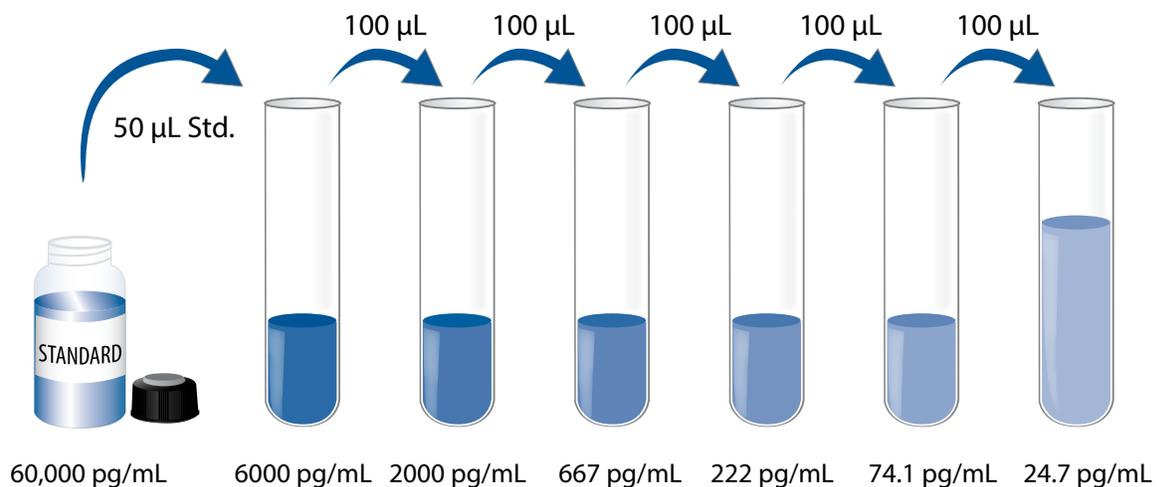
Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 μ L of the resultant mixture is required per well.

Aldosterone Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Aldosterone Standard with deionized or distilled water. This reconstitution produces a stock solution of 60,000 pg/mL. **Allow the standard to sit for a minimum of 60 minutes with gentle mixing prior to making dilutions.**

Pipette 450 μ L of Calibrator Diluent RD5-69 into the 6000 pg/mL tube. Pipette 200 μ L into the remaining tubes. Use the stock solution to produce a 3-fold dilution series (below). Mix each tube gently but thoroughly before the next transfer. The 6000 pg/mL standard serves as the high standard. Calibrator Diluent RD5-69 serves as the zero standard (0 pg/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples, controls, and standards be assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 100 μL of Aldosterone Primary Antibody Solution to each well (**excluding the NSB wells**). Cover with the adhesive strip provided. Incubate for 1 hour at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm.
4. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 μL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
5. Add 50 μL of Assay Diluent RD1-120 to each well.
6. Add 75 μL of Standard, control, or sample* to the appropriate wells.
7. Add 75 μL of Calibrator Diluent RD5-69 to the zero standard (B_0) wells and NSB wells.
8. **Briefly vortex Aldosterone Conjugate prior to use.** Add 50 μL of the Aldosterone Conjugate to all wells. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature on the shaker.
9. Repeat the aspiration/wash as in step 4.
10. Add 200 μL of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
11. Add 100 μL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

*Samples require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample then subtract the average NSB optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on a linear y-axis against the concentration on a logarithmic x-axis and draw the best fit curve through the points on the graph. Do not include the B_0 in the standard curve.

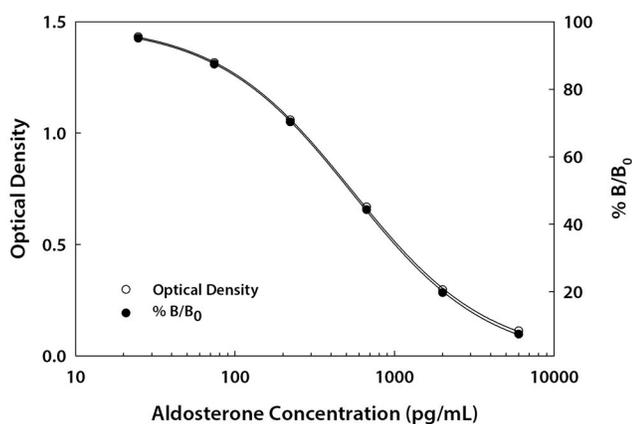
If desired, % B/ B_0 can be calculated by dividing the corrected O.D. for each standard or sample by the corrected B_0 O.D. and multiplying by 100.

Calculate the concentration of Aldosterone corresponding to the mean absorbance from the standard curve.

Since samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D.	Average	Corrected	% B/ B_0
NSB	0.016 0.018	0.017	—	—
0 (B_0)	1.484 1.561	1.523	1.506	—
24.7	1.448 1.450	1.449	1.432	95
74.1	1.326 1.339	1.333	1.316	87
222	1.070 1.082	1.076	1.059	70
667	0.674 0.695	0.685	0.668	44
2000	0.313 0.315	0.314	0.297	20
6000	0.128 0.129	0.129	0.112	7

PRECISION

Intra-assay Precision (Precision within an assay)

Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)

Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of kit components.

Sample	Intra-Assay Precision			Inter-Assay Precision		
	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	278	878	2536	263	832	2419
Standard deviation	9.49	17.3	34.4	31.7	83.0	230
CV (%)	3.4	2.0	1.4	12.1	10.0	9.5

RECOVERY

The recovery of Aldosterone spiked to levels throughout the range of the assay in various matrices was evaluated. Samples were diluted prior to assay as directed in the Sample Preparation section.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	94	89-99%
Serum (n=4)	88	78-108%
EDTA plasma (n=4)	89	76-110%
Heparin plasma (n=4)	89	79-107%
Urine (n=4)	101	95-106%

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of Aldosterone were diluted with Calibrator Diluent to produce samples with values within the dynamic range of the assay. Samples were diluted prior to assay as directed in the Sample Preparation section.

		Cell culture media (n=4)	Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)	Urine (n=4)
1:2	Average % of Expected	107	108	107	109	101
	Range (%)	105-108	100-114	100-113	97-115	98-106
1:4	Average % of Expected	109	111	110	109	98
	Range (%)	105-112	97-119	96-120	93-117	95-103
1:8	Average % of Expected	99	102	100	103	98
	Range (%)	95-104	82-111	79-118	83-116	89-111

SENSITIVITY

Twenty-Six assays were evaluated and the minimum detectable dose (MDD) of Aldosterone ranged from 7.74-22.4 pg/mL. The mean MDD was 15.2 pg/mL.

The MDD was determined by subtracting two standard deviations from the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of Aldosterone in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Serum (n=36)	258	97	ND-849
EDTA plasma (n=36)	280	92	ND-828
Heparin plasma (n=36)	226	81	ND-828

ND=Non-detectable

Sample Type	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Urine (n=10)	8222	1900-22,191	6973

Cell Culture Supernates - Human peripheral blood lymphocytes (1×10^6 cells) were cultured in RPMI 1640 supplemented with 10% fetal bovine serum. Cells were cultured unstimulated or stimulated with 10 μ g/mL PHA for 1 and 5 days. Aliquots of the cell culture supernates were removed and assayed for levels of Aldosterone. No detectable levels were observed.

SPECIFICITY

The factors listed below were prepared at 50 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range Aldosterone control were assayed for interference. No significant cross-reactivity or interference was observed.

Substances:

Androsterone	Estriol
Canrenic Acid	Estrone
Corticosteroid Binding Globulin	Prednisolone
Cortisol	Prednisone
Deoxycorticosterone	Progesterone
Dexamethasone	Spirolactone
Eplerenone	Testosterone
Estradiol	

Corticosterone cross-reacts approximately 0.07% in this assay.

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