Product Manual

# Aldosterone ELISA Kit

Catalog #: ADI-901-173

480 Well Kit

For use with plasma, serum, and urine

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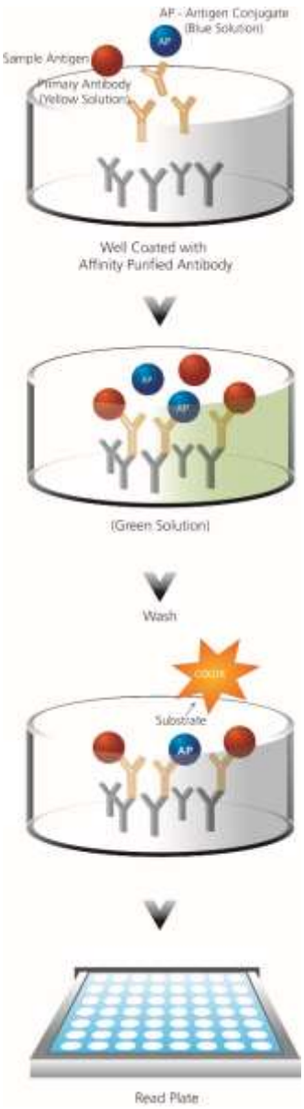
# Product Manual

## TABLE OF CONTENTS

Please read entire booklet before proceeding with the assay.

Carefully note the handling and storage conditions of each kit component.

Please contact Enzo Life Sciences Technical Support if necessary.




## INTRODUCTION

The Enzo Life Sciences Aldosterone Enzyme Immunoassay (ELISA) Kit is a complete kit for the quantitative determination of aldosterone in plasma, serum, and urine samples. Please read the complete kit insert before performing the assay.

Aldosterone is a steroid hormone synthesized from cholesterol in the adrenal cortex. Aldosterone is metabolized in the kidney and liver, and functions as the key mineralocorticoid in the control of sodium and potassium balance. Synthesis and release of aldosterone by the adrenal gland is primarily regulated by the renin-angiotensin-alsdosterone system (RAAS), the main regulatory system involved in blood pressure regulation, renal hemodynamics, and sodium-volume homeostasis[1]. Measurement of serum aldosterone in conjunction with plasma renin is used clinically to differentiate between primary and secondary aldosteronism[2]. Primary aldosteronism (hyperaldoseronism) is characterized by a very low renin:aldosteone ratio leading to the retention of sodium and increased blood pressure, and is typically the result of renal gland hyperplasia or tumors. In secondary aldosteronism, hyperproduction of aldosterone results from external conditions such as heart failure and renal artery disease that reduce renal blood flow and stimulate the RAAS mechanism[3].

The RAAS directly affects vascular and cardiac remodeling through proliferative and inflammatory signaling, as aldosterone and salt have been shown to increase the expression of intracellular cyclooxygenase-2, osteopontin, and MCP-1 in rats[1,4]. Aldosterone acts by binding to the mineralocorticoid receptor (MR) triggering the transcription of hormone responsive genes, and clinical studies have shown that patients with congestive heart failure or after myocardial infarction benefited from MR antagonist treatment[5]. As pharmacological modulation of nuclear hormone receptors is a common strategy for the treatment of cardiovascular disease, determining the effect of such treatments on the RAAS is of increasing value in evaluating the safety and efficacy of new targeted therapeutics[1].

## PRINCIPLE

1. Standards and samples are added to wells coated with a donkey anti-sheep IgG antibody. A blue solution of aldosterone conjugated to alkaline phosphatase is then added, followed by a yellow solution of sheep polyclonal antibody to aldosterone.
2. During a simultaneous incubation at 4°C the antibody binds, in a competitive manner, the aldosterone in the sample or conjugate. The plate is washed, leaving only bound aldosterone.
3. pNpp substrate solution is added. The substrate generates a yellow color when catalyzed by the alkaline phosphatase on the aldosterone conjugate.
4. Stop solution is added. The yellow color is read at 405 nm. The amount of signal is indirectly proportional to the amount of aldosterone in the sample.

## MATERIALS SUPPLIED

1. **Assay Buffer, 27 mL Catalog No. 80-0011:**

   Tris buffer containing proteins and sodium azide

2. **Aldosterone Standard, 2 x 0.25 mL, Catalog No. 80-2071**

   A solution of 10,000 pg/mL aldosterone

3. **Donkey anti-Sheep IgG Microtiter Plate, Five plates of 96 wells, Catalog No. 80-0045**

   A clear plate of break-apart strips coated with a donkey anti-sheep polyclonal antibody

4. **Aldosterone ELISA Antibody, 25 mL, Catalog No. 80-2086**

   A yellow solution of sheep polyclonal antibody to aldosterone

5. **Aldosterone ELISA Conjugate, 25 mL Catalog No. 80-2087**

   A blue solution of aldosterone conjugated to alkaline phosphatase

6. **Wash Buffer Concentrate, 100 mL, Catalog No. 80-1287**

   Tris buffered saline containing detergents

7. **pNpp Substrate, 100 mL, Catalog No. 80-0076**

   A solution of p-nitrophenyl phosphate

8. **Stop Solution, 27 mL, Catalog No. 80-0248**

   A solution of trisodium phosphate in water

9. **Aldosterone Assay Layout Sheet, Catalog No. 30-0280**

   1 each

10. **Plate Sealer, Catalog No. 30-0012**

    5 each

Do not mix components from different kit lots or use reagents beyond the expiration date of the kit.

The standard should be handled with care due to the known and unknown effects of the antigen.

Avoid contamination by endogenous alkaline phosphatase. Do not expose reagents or supplies to bare skin.

Activity of conjugate is affected by nucleophiles such as azide, cyanide, and hydroxylamine.

Stop solution is caustic. Keep tightly capped.

Reagents require separate storage conditions.

## STORAGE

All components of this kit are shipped at -20°C.

Upon receipt, the kit components can be stored at the temperature indicated on the component label or the whole kit can continue to be stored at -20°C until first use.

## OTHER MATERIALS NEEDED

1. Deionized or distilled water
2. Precision pipets for volumes between 5 µL and 1,000 µL
3. Repeater pipet for dispensing 50 µL and 200 µL
4. Disposable beakers for diluting buffer concentrates
5. Graduated cylinders
6. Lint-free paper toweling for blotting
7. Microplate reader capable of reading at 405 nm
8. Data reduction software capable of analyzing data, preferably with a 4 parameter logistic curve fit
9. Additional materials may be required for sample preparation. Please see Sample Handling section for details.

## SAMPLE HANDLING

Serum and plasma samples may be diluted in Assay Buffer and run directly in the assay. A minimum 1:8 dilution is required for human serum. A minimum 1:4 dilution is required for rat serum, and human or rat plasma. Urine samples may be run in the assay after acid hydrolysis, with a minimum required dilution of 1:16. These are the minimum dilutions required to remove matrix interference of these samples (see the Sample Recoveries section).

Some samples may contain high levels of aldosterone and additional dilution may be required. Samples with low levels of aldosterone may be concentrated and assayed by first performing a solid phase extraction.

### Protocol for Urine Samples

#### Acid Hydrolysis

**Materials Needed:**

1. 0.2N HCl
2. Tube with lid/bottle sufficient for urine volume

**Procedure:**

1. Centrifuge urine at ~20,000 x g for 5 minutes at 4°C.
2. Transfer 1 part supernatant to a tube/bottle, and add 2 parts of 0.2 N HCl. (e.g. 500 µL urine + 1 mL 0.2 N HCl)
3. Leave overnight at room temperature, capped and protected from light.
4. Samples may now be stored at -20°C or assayed immediately.

## Protocol for Serum/Plasma Samples (optional)

### Solid phase extraction

**Materials Needed:**

1. Aldosterone standard to allow extraction efficiency to be accurately determined. An extraction efficiency protocol is available on our website at www.enzolifesciences.com
2. 1000 mg $C_{18}$ solid phase system columns (Burdick & Jackson recommended)
3. Vacuum manifold
4. Speedvac
5. 100% Methanol
6. Diethyl ether
7. $dH_20$

**Procedure:**

1. Condition 1000 mg $C_{18}$ solid phase system columns on a vacuum manifold by passing 5-10 mL of 100% methanol through the columns, followed by 5-10 mL of $dH_2O$.
2. Apply serum and plasma samples.
3. Wash columns with 5-10 mL $dH_2O$. Allow water to drain completely from columns until dry.
4. Elute samples with 2 mL of diethyl ether.
5. Dry samples down in a speedvac for 2-3 hrs.
6. Rehydrate samples at room temperature in the assay buffer. A minimum of 250 µL of the assay buffer is recommended for reconstitution to allow for duplicate sample measurement.

## SAMPLE RECOVERIES

After diluting each sample matrix, aldosterone standard was spiked at high, medium, and low concentrations. The recovery of the standard in spiked samples was compared to the recovery of identical spikes in the assay buffer. The mean of percent recovery at the three concentrations are indicated below for each matrix.

| Sample | Dilution | Spike Concentration [pg/mL] | Mean % Recovery |
|---|---|---|---|
| Human serum | 1:16 | 120.0 | 75.7% |
| | | 30.0 | 76.1% |
| | | 7.5 | 57.6% |
| Human plasma | 1:8 | 120.0 | 88.1% |
| | | 30.0 | 112.5% |
| | | 7.5 | 124.2% |
| Human urine | 1:32 | 120.0 | 124.8% |
| | | 30.0 | 94.2% |
| | | 7.5 | 77.1% |

Bring all reagents to room temperature for at least 30 minutes prior to opening.

Glass or polypropylene tubes may be used for standard preparation. Avoid polystyrene.

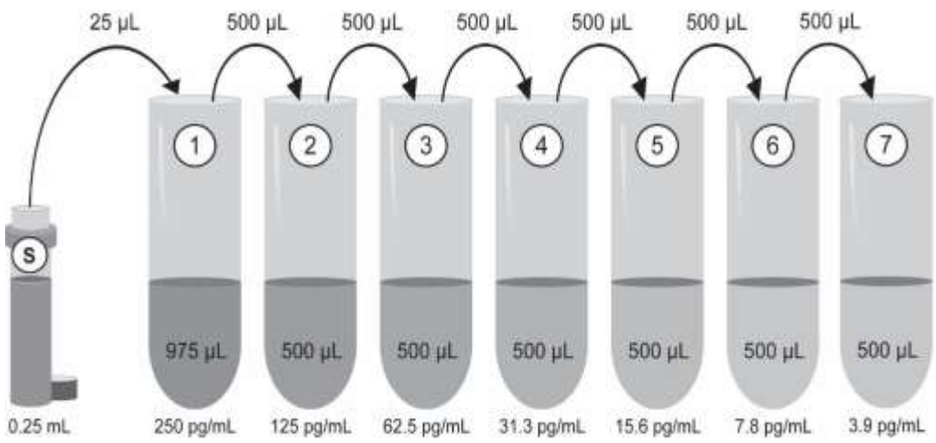
## REAGENT PREPARATION

1. **Assay Buffer**

   Prepare the Assay Buffer by diluting 10 mL of the supplied Wash Buffer Concentrate with 90 mL of deionized water. This can be stored at room temperature until the kit expiration, or for 3 months, whichever is earlier.

2. **Aldosterone Standard**

   Allow the 10,000 pg/mL aldosterone standard to come to room temperature prior to use. Label seven 12 x 75mm tubes #1 through #7. Pipet 975 µL of the assay buffer into tube #1. Pipet 500 µL of the assay buffer into tubes #2 through #7. Remove 25 µL from the 10,000 pg/mL aldosterone stock vial and add to tube #1. Vortex thoroughly. Remove 500 µL from tube #1 and add to tube #2. Vortex thoroughly. Continue this for tubes #3 through #7.




   **Diluted standards should be used within 60 minutes of preparation.** The concentrations of aldosterone in the tubes are labeled above.

3. **Conjugate 1:5 Dilution for Total Activity Measurement**

   Prepare the Conjugate 1:5 Dilution by diluting 20 µL of the supplied Conjugate with 80 µL of the assay buffer. The dilution should be made after the overnight incubation. **This 1:5 dilution is intended for use in the Total Activity wells ONLY.**

4. **Wash Buffer**

   Prepare the wash buffer by diluting 5 mL of the supplied Wash Buffer Concentrate with 95 mL of deionized water. This can be stored at room temperature until the kit expiration, or for 3 months, whichever is earlier.

## ASSAY PROCEDURE

Refer to the Assay Layout Sheet to determine the number of wells to be used. Remove the wells not needed for the assay and return them, with the desiccant, to the mylar bag and seal. Store unused wells at 4°C.

Bring all reagents to room temperature for at least 30 minutes prior to opening.

1. Pipet 150 µL of the assay buffer into the NSB (non-specific binding) wells.
2. Pipet 100 µL of the assay buffer into the Bo (0 pg/mL standard) wells.
3. Pipet 100 µL of Standards #1 through #7 to the bottom of the appropriate wells.
4. Pipet 100 µL of the samples to the bottom of the appropriate wells.
5. Pipet 50 µL of the blue conjugate into each well except the TA and Blank wells.
6. Pipet 50 µL of the yellow antibody into each well except the Blank, TA, and NSB wells.

   **Note**: Every well used should be green in color except the NSB wells which should be blue. The Blank and TA wells are empty at this point and have no color.
7. Seal the plate. Incubate for overnight (16-24 hours) at 4°C hours, no shaking.
8. Empty the contents of the wells and wash by adding 400 µL of wash buffer to every well. Repeat 2 more times for a total of **3 washes**. After the final wash, empty or aspirate the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.
9. Pipet 5 µL of the blue conjugate (diluted 1:5) to the TA wells.
10. Add 200 µL of the substrate solution into each well.
11. Incubate for 1 hour at room temperature without shaking.
12. Pipet 50 µL stop solution into each well.
13. After blanking the plate reader against the substrate blank, read optical density at 405 nm. If plate reader is not capable of adjusting for the blank, manually subtract the mean OD of the substrate blank from all readings.

Pre-rinse each pipet tip with reagent. Use fresh pipet tips for each sample, standard, and reagent.

All standards and samples should be run in duplicate.

Pipet the reagent to the side of the wells to avoid possible contamination.

Prior to the addition of the substrate, ensure there is no residual wash buffer in the wells. Remaining wash buffer may cause variation in assay results.

## CALCULATION OF RESULTS

Make sure to multiply sample concentrations by the dilution factor used during sample preparation.

Several options are available for the calculation of the concentration of aldosterone in the samples. We recommend that the data be handled by an immunoassay software package utilizing a four parameter logistic curve fitting program (such as AssayBlaster™, catalog number ADI-28-0002). Such software is often supplied by plate reader manufacturers. If data reduction software is not readily available, the concentration of aldosterone can be calculated as follows:

1. Calculate the average net OD for each standard and sample by subtracting the average NSB OD from the average OD for each standard and sample.

   **Average Net OD = Average OD - Average NSB OD**

2. Calculate the binding of each pair of standard wells as a percentage of the maximum binding wells (Bo), using the following formula:

$$\text{Percent Bound} = \frac{\text{Net OD}}{\text{Net Bo OD}} \times 100$$

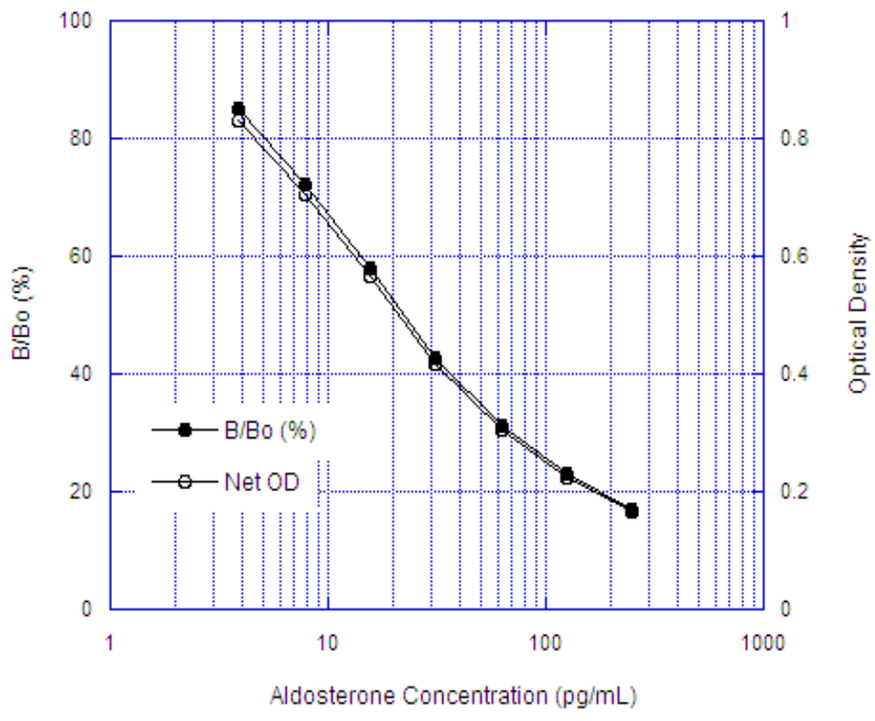
3. Plot the Percent Bound (B/Bo) versus concentration of Aldosterone for the standards. Approximate a straight line through the points. The concentration of Aldosterone of the unknowns can be determined by interpolation.

Samples with concentrations outside of the standard curve range will need to be reanalyzed using a different dilution.

## TYPICAL RESULTS

**The results shown below are for illustration only and should not be used to calculate results from another assay.**

| Sample | Average Net OD | Percent Bound | Aldosterone (pg/mL) |
|---|---|---|---|
| TA | 2.528 | --- | --- |
| NSB | 0.001 | 0% | --- |
| Bo | 1.047 | 100% | **0** |
| S1 | 0.199 | 19.0% | **250** |
| S2 | 0.258 | 24.6% | **125** |
| S3 | 0.346 | 33.0% | **62.5** |
| S4 | 0.472 | 45.0% | **31.3** |
| S5 | 0.671 | 64.0% | **15.6** |
| S6 | 0.776 | 74.1% | **7.8** |
| S7 | 0.905 | 86.4% | **3.9** |
| Unknown 1 | 0.301 | 28.8% | **83.8** |
| Unknown 2 | 0.762 | 72.8% | **9.3** |



## PERFORMANCE CHARACTERISTICS

### Specificity

The cross reactivities for a number of related compounds were determined by diluting the cross reactants in the kit assay buffer at a concentration of 100, 10, 1, and 0.1 times the high standard. These samples were then measured in the assay.

| Compound | %XR |
|---|---|
| 11-Deoxycorticosterone | 0.30% |
| Progesterone | 0.20% |
| Corticosterone | 0.19% |
| Cortisol | ≤ 0.001% |
| DHT | ≤ 0.001% |
| Estradiol | ≤ 0.001% |
| Testosterone | ≤ 0.001% |

### Sensitivity

The sensitivity or limit of detection of the assay is 4.7 pg/mL. The sensitivity was determined by interpolation at 2 standard deviations below the mean signal at maximal binding (0 ng/mL) using data from 13 standard curves.

### Linearity

Human and rat samples containing Aldosterone were serially diluted 1:2 in the kit assay buffer and measured in the assay. The results are shown in the table below.

| | Average % of Expected | | | | |
|---|---|---|---|---|---|
| Dilution | Human Plasma | Rat Plasma | Rat Serum | Human Serum | Human Urine |
| Neat | --- | --- | 108% | --- | --- |
| 1:2 | 89% | 105% | 108% | --- | --- |
| 1:4 | 95% | 102% | 109% | --- | --- |
| 1:8 | 92% | 84% | 121% | 93% | --- |
| 1:16 | 100% | 86% | 122% | 96% | 107% |
| 1:32 | --- | 98% | 90% | 98% | 103% |
| 1:64 | --- | 109% | 103% | 100% | 100% |

## Precision

**Intra-assay precision** was determined by assaying 20 replicates of three buffer controls containing Aldosterone in a single assay.

| pg/mL | %CV |
|---|---|
| 84.8 | 4.5 |
| 27.7 | 4.4 |
| 13.9 | 6.6 |

**Inter-assay precision** was determined by measuring buffer controls (n=13) of varying Aldosterone concentrations in multiple assays over several days.

| pg/mL | %CV |
|---|---|
| 72.6 | 10.8 |
| 24.4 | 18.0 |
| 10.8 | 16.3 |

## REFERENCES


1. Kuipers, I., et al. (2008) Hypertension **51**, 1442-1448.
2. Tiu, S-C., et. al. (2005) J Clin Endocrinol Metab. **90**, 72–8.
3. Merck Online Medical Library (2007) Adrenal Disorders: Secondary Aldosteronism. Available at www.merck.com/mmpe/sec12/ch153/ch153g.html (Accessed June 2009).
4. Rocha, R., et al. (2002) Am J Physiol Heart Circ Physiol. **283**, H1802-H1810.
5. Grossmann, C. and Gekle, M. (2009) Molecular and Cellular Endocrinology **308**, 53-62.

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