Aldosterone Competitive ELISA Kit

Catalog Number EIAALD (96 tests)

Rev 1.0

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *ELISA Technical Guide* (Pub. no. MAN0006706). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The Aldosterone Competitive ELISA Kit is a solid-phase competitive Enzyme-Linked Immunosorbent Assay (ELISA). This assay is designed to detect and quantify the aldosterone in extracted serum, EDTA or heparin plasma, and urine, fecal extracts, and tissue culture media. The assay recognizes aldosterone independent of species.

Contents and storage

Kit and components are shipped at –20°C. Upon receipt, store the kit at –20°C. Once open, store the kit at 4°C and use within 2 weeks.

Components	Quantity
Aldosterone Standard; 40,000 pg/mL aldosterone in a special stabilizing solution	125 µL
Assay Buffer Concentrate (5X)	28 mL
Antibody Coated Wells, 96-well strip-well plate coated with goat anti-rabbit IgG	1 plate
Aldosterone Antibody	3 mL
Aldosterone Conjugate	3 mL
Wash Buffer Concentrate (20X)	30 mL
TMB (Tetramethylbenzidine) Substrate	11 mL
Stop Solution; contains 1 M HCl, **CAUSTIC**	5 mL
Plate Sealer	1

Materials required but not supplied

- Distilled or deionized water
- Microtiter plate reader with software capable of measurement at or near 450 nm (preferably with correction between 570 nm and 590 nm).
- Plate washer–automated or manual (squirt bottle, manifold dispenser, or equivalent)
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solution

Procedural guidelines

IMPORTANT! Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

- Review the **Procedural guidelines** and **Plate washing directions** in the *ELISA Technical Guide* available at **thermofisher.com**.
- Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.
- Solutions containing sodium azide will inhibit the activity of the peroxidase conjugate. Ensure that there is no contamination of labware or the plate washer with azide containing solutions.

Prepare 1X Wash Buffer

1. Dilute 15 mL of Wash Solution Concentrate (20X) with 285 mL of deionized or distilled water. Label as 1X Wash Buffer.
2. Store the concentrate and 1X Wash Buffer in the refrigerator. Use the diluted buffer within 3 months.

Prepare 1X Assay Buffer

1. Dilute 14 mL of Assay Buffer (5X) with 56 mL of deionized or distilled water. Label as 1X Assay Buffer.
2. Store the concentrate and 1X Assay Buffer in the refrigerator. 1X Assay Buffer is stable at 4°C for 3 months.

Sample preparation guidelines

- Refer to the *ELISA Technical Guide* at **thermofisher.com** for detailed sample preparation procedures.
- Collect samples in pyrogen/endotoxin-free tubes.
- Freeze samples after collection if samples will not be tested immediately. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well (do not vortex) prior to analysis.
- Avoid the use of hemolyzed or lipemic sera.
- If large amounts of particulate matter are present in the sample, centrifuge or filter sample prior to analysis.

Prepare samples

Sample concentrations should be within the range of the standard curve. Because conditions may vary, each investigator should determine the optimal dilution for each application.

Use all samples within **2 hours** of dilution, or store at –20°C or lower until ready to perform assay.

Sample type	Procedure
Serum and plasma	1. Add 250 µL of serum or plasma to a glass test tube and add 250 µL of ethyl acetate. 2. Vortex gently and allow layers to separate. Gently draw off the top organic layer and place it in a clean tube. 3. Repeat the extraction with ethyl acetate 2 more times, pooling the ethyl acetate supernatants. 4. Speedvac the ethyl acetate supernatant to dryness. 5. Reconstitute with 10 µL of ethanol and dilute with 240 µL of 1X Assay Buffer. This dilution can be diluted further with Assay Buffer.
Urine	Dilute samples ≥1:4 with 1X Assay Buffer. **Note**: A Urinary Creatinine Detection Kit (Cat. no. EIACUN) is available for measuring urine creatinine for normalization of aldosterone in a random urine specimens.
Dried feces	See detailed extraction protocol on the product page at **thermofisher.com** **Note**: The ethanol concentration in the final diluted Assay Buffer dilution added to the well should be <5%.
Tissue culture media	Perform sample dilutions with the corresponding tissue culture medium.

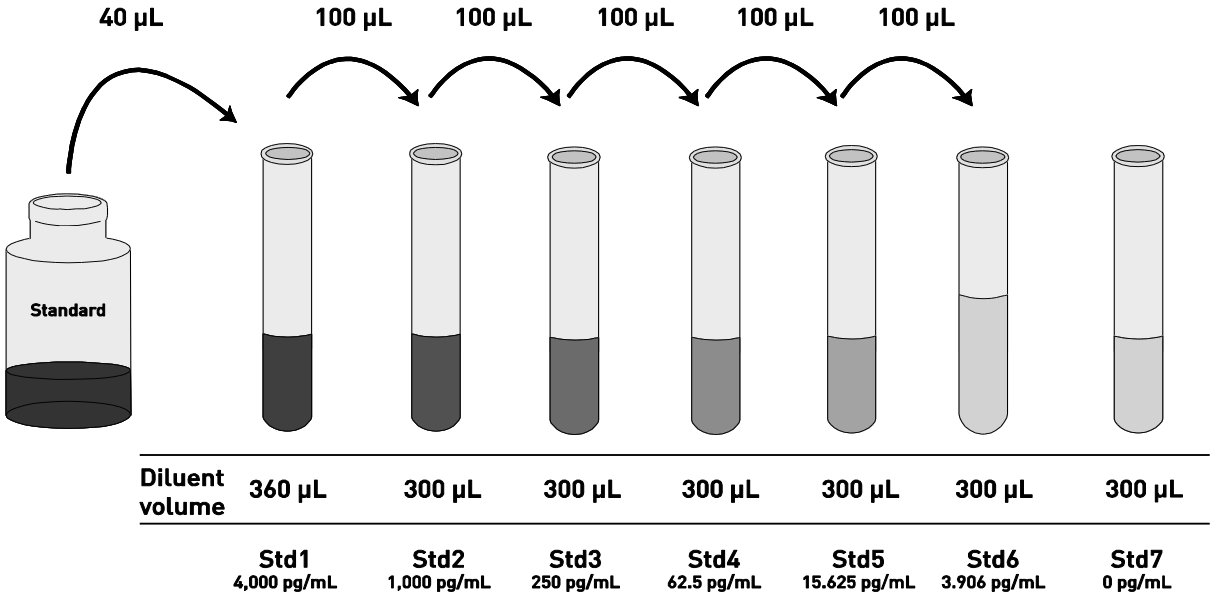
Dilute standards

Note: Use glass or plastic tubes for diluting standards.

Instructions are for diluting standards from 4,000 to 3.906 pg/mL, but a curve can be obtained using a range of 1,000 to 3.906 pg/mL. Choose the range that fits your sample concentrations most appropriately.

The Aldosterone Standard contains an organic solvent. Pipette the standard up and down several times to wet the pipet tip before transfer to ensure that volumes are accurate.

1. Add 40 µL Aldosterone Standard to one tube containing 360 µL 1X Assay Buffer and label as 4,000 pg/mL aldosterone.
2. Add 300 µL of 1X Assay Buffer to each of 6 tubes labeled as follows: 1,000; 250; 62.25; 15.625; 3.906; and 0 pg/mL aldosterone.
3. Make serial dilutions of the standard as described below in the dilution diagram. Mix thoroughly between steps.
4. **Use the standards within 2 hours of preparation**.

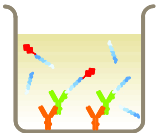


Perform ELISA (Total assay time: 12-16 hours)

IMPORTANT! Perform a standard curve with each assay.

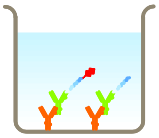
Allow all components to reach room temperature before use. Mix all liquid reagents prior to use.

Determine the number of 8-well strips required for the assay. Insert the strips in the frames for use. Re-bag any unused strips and frames, and store desiccated at 2°C to 8°C for future use. The silica pack in the bag keeps the plate dry, and turns from blue to pink if the bag is not properly sealed.



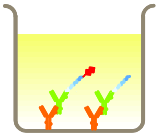
Bind antigen

a. Add 100 µL of standards or samples (see "Prepare samples" on page 2) to the appropriate wells.
b. Add 125 µL of 1X Assay Buffer into wells for detecting non-specific binding (NSB).
c. Add 25 µL of Aldosterone Conjugate to each well.
d. Add 25 µL of Aldosterone Antibody to each well except NSB wells.
e. Tap the side of the plate to mix. Cover the plate with plate sealer and incubate for 15 minutes at room temperature with shaking.
f. Store the sealed plate at 4°C overnight.
g. Thoroughly aspirate the solution and wash wells 4 times with 300 µL of 1X Wash Buffer.



Add chromogen

a. Add 100 µL TMB Substrate to each well. The substrate solution will begin to turn blue.
   Note: Addition of cold TMB Substrate will cause depressed signal.
b. Incubate for 30 minutes at room temperature without shaking.
   Note: TMB should not touch aluminum foil or other metals.



Add stop solution

Add 50 µL Stop Solution to each well. Tap side of the plate gently to mix. The solution in the wells changes from blue to yellow.

Read the plate and generate the standard curve

1. Read the absorbance at 450 nm. Read the plate within 10 minutes after adding the Stop Solution.
2. Use curve-fitting software to generate the standard curve. A four parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.
3. Read the concentrations for unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.

   Note: Dilute samples producing signals lower than that of the highest standard and reanalyze. Multiply the concentration by the appropriate dilution factor.

Performance characteristics

Standard curve (example)

The following data were obtained for the various standards over the range of 0–4,000 pg/mL aldosterone.

Standard Aldosterone (pg/mL)	Optical Density (450 nm)*
4,000	0.202
1,000	0.318
250	0.497
62.25	0.751
15.625	0.904
3.906	0.993
0	1.024

Note: The NSB gave a Mean OD value of 0.063.

Intra-assay precision

Samples were assayed in replicates of 20 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	1,018.7	156.2	40.6
%CV	6.0	5.9	8.8

CV = Coefficient of Variation

Inter-assay precision

Samples were assayed in duplicates in 17 assay runs by four operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	1,051.9	150.2	39.6
%CV	20.5	12.2	25.8

CV = Coefficient of Variation

Performance characteristics, continued

Expected values

Thirteen random mammalian serum and plasma samples were tested in the assay. Neat EDTA plasma sample values ranged from 9.98 to 66.7 pg/mL with an average of 33.0 pg/mL. Serum values ranged from 15.9 to 147.0 pg/mL with an average of 77.8 pg/mL. Nine human urine samples were tested in the assay and the values ranged from 431.9 to 5,114 pg/mL with an average of 2267.4 pg/mL. One dog urine sample was tested and it read at 10,079 pg/mL.

Dried clouded leopard fecal samples were processed and run in the assay. Values obtained ranged from 19.4 to 22.8 pg/mg dried fecal material.

Recovery

Recovery was determined by taking two urine samples, one with a low diluted aldosterone level of 43.0 pg/mL and one with a higher diluted level of 1,001.7 pg/mL, and mixing them in the ratios given below. The measured concentrations were compared to the expected values based on the ratios used.

Low Sample %	High Sample %	Expected Conc. (pg/mL)	Observed Conc. (pg/mL)	% Recovery
80	20	234.7	187.6	79.9
60	40	426.5	501.4	117.6
40	60	618.2	614.5	99.4
20	80	810.0	856.7	105.8
			Mean Recovery	**100.7%**

Specificity

The following cross reactants were tested in the assay and calculated at the 50% binding point.

Steroid	Cross-reactivity %
Aldosterone	100
Corticosterone	0.047
Desoxycorticosterone	0.019
Progesterone	<0.016
Tetrahydrocorticosterone	<0.016
Cortisol	<0.016
1-dehydrocortisol	<0.016
Estradiol	<0.016

Sensitivity

The analytical sensitivity of aldosterone is 4.97 pg/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Limited product warranty

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REF Catalog Number | LOT Batch code | Temperature limitation | Use by | Manufacturer | Consult instructions for use | Caution, consult accompanying documents

Manufacturer's address: Life Technologies Corporation | 7335 Executive Way | Frederick, MD 21704 | USA

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