ab285301 – Aldosterone ELISA Kit

For *in vitro* quantitative determination of Aldosterone.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab285301

Storage and Stability

On receipt entire assay kit should be stored at 4°C. Upon opening, use kit within 6 months.

Materials Supplied

Item	Quantity	Storage Condition
Micro ELISA Plate	8 x 12 strips	4°C
Standard	125 µl	4°C
Aldosterone Antibody	3 ml	4°C
Aldosterone Conjugate	3 ml	4°C
Assay Buffer Concentrate (5X)	28 ml	4°C
Wash Buffer Concentrate (20X)	30 ml	4°C
TMB Substrate	11 ml	4°C
Stop Solution	5 ml	4°C
Plate Sealer	1 unit	4°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450 nm
- Ethyl acetate or ethanol for serum, plasma, or fecal extracts
- Speedvac for evaporation of ethanol or ethyl acetates
- Precision pipettes with disposable tips

Reagent Preparation

- Prepare reagents within 30 minutes before the experiment.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.

Assay Buffer: Dilute Assay Buffer Concentrate 1:5 by adding one part of the concentrate to four parts of deionized water. Once diluted this is stable at 4°C for 3 months.

Wash Buffer: Dilute Wash Buffer Concentrate 1:20 by adding one part of the concentrate to nineteen parts of deionized water. Once diluted this is stable for 3 months at room temperature.

Standard Preparation:

1. Add 40 µl of the aldosterone stock solution to 360 µl of Assay Buffer (tube #1) and vortex completely.
2. Prepare 4 vials of standards (tube #2-6) by adding 0.1 ml of the above stock solution in 0.3 ml of Assay Buffer. Perform 4-fold serial dilutions of the top standards to make the standard curve within the range of this assay.
3. Suggested standard points are: 4,000, 1,000, 250, 62.5, 15.6, and 3.9 pg/ml.

Sample Preparation:

- Use all Samples within 2 hours of preparation or stored at ≤ -20°C until assaying.
- Avoid multiple freeze-thaw cycles.
- End user should estimate the concentration of the target protein in the test sample first and select a proper dilution factor to make the diluted target protein concentration fall in the optimal detection range of the kit.

Serum: Add 250 µl of serum or plasma to a glass test tube and add 250 µl of ethyl acetate. Vortex gently and allow layers to separate. Gently draw off the top organic layer and place it in a clean tube. Repeat the extraction with ethyl acetate 2 more times, pooling the ethyl acetate supernatants. Speedvac the ethyl acetate supernatant to dryness. Reconstitute with 10 µl of ethanol and dilute with 240 µl of supplied Assay Buffer. This dilution can be diluted further with Assay Buffer.
Urine: Urine samples should be diluted ≥ 1:4 with the supplied Assay Buffer prior running in the assay.
Tissue Culture Media: For measuring aldosterone in tissue culture media (TCM), samples should be read off a standard curve generated in TCM. Samples may need to be diluted further in TCM.

Assay Protocol

- A Standard Curve must be run with each assay.
- Bring all Buffer(s) to RT 30 minutes prior to the assay.
- It is recommended that all standards and samples be run at least in duplicate.

1. Prepare all reagents, samples and standards as instructed.
2. Pipet 100 µl of samples or standards into wells in the plate. Pipet 125 µl of Assay Buffer into the non-specific binding (NSB) wells.
3. Add 25 µl of the Aldosterone Conjugate to each well. Add 25 µl of the Aldosterone Antibody to each well, except the NSB wells.
4. Gently tap the sides of the plate to ensure adequate mixing of the reagents. Cover the plate with the plate sealer and shake at room temperature for 15 minutes. Store the sealed plate at 4°C overnight.
5. The next day, bring TMB substrate to room temperature 30 minutes prior to the assay.
6. Aspirate the plate and wash each well 4 times with 300 µl wash buffer. Tap the plate dry on clean absorbent towels.
7. Add 100 µl of the TMB Substrate to each well. Incubate the plate at room temperature for 30 minutes.
8. Add 50 µl of the Stop Solution to each well.
9. Read the optical density at 450 nm within 15 minutes.

Calculation:

1. Average the duplicate OD readings for each standard and sample.
2. Create a standard curve by reducing the data using the 4PLC fitting routine on the plate reader, after subtracting the mean OD's for the non-specific binding well (NSB).
3. The sample concentrations obtained, calculated from the %B/B0 curve, and should be multiplied by the dilution factor to obtain neat sample values.

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)