ab285306 – Angiotensin II (Ang II) ELISA Kit

For the quantitative measurement of Ang II in human serum, plasma, culture supernatants, Tissue homogenates and other biological fluids.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab285306

Storage and Stability

The entire ELISA kit may be stored at 4°C for up to 6 months from the date of shipment.

Materials Supplied

Item	Quantity	Storage Condition
Micro ELISA Plate	8 x 12 wells	4°C
Lyophilized Standard 2000 pg	2 vials	4°C
Sample / Standard dilution buffer	20 mL	4°C
Biotin-detection antibody (Concentrated) (Avoid Light)	60 µL	4°C
Antibody dilution buffer	10 mL	4°C
HRP-Streptavidin Conjugate (SABC) (Avoid Light)	120 µL	4°C
SABC dilution buffer	10 mL	4°C
TMB substrate (Avoid Light)	10 mL	4°C
Stop Solution	10 mL	4°C
Wash buffer (25X)	30 mL	4°C
Plate sealers	5 units	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
- 37°C incubator
- Precision pipettes with disposable tips
- Clean eppendorf tubes for preparing standards or sample dilutions
- Absorbent paper

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.

Biotin-detection antibody working solution: Calculate the total volume of the working solution: 0.05 ml / well × quantity of wells with additional 0.1 - 0.2 ml of the total volume. Dilute the Biotin-detection antibody with Antibody dilution buffer at 1:100 and mix thoroughly.

HRP-Streptavidin Conjugate (SABC): Calculate the total volume of the working solution: 0.1 ml / well × quantity of wells with additional 0.1 - 0.2 ml of the total volume. Dilute the SABC with SABC dilution buffer at 1:100 and mix thoroughly.

Wash Buffer: Dilute 30 mL of Concentrated Wash Buffer into 750 mL of Wash Buffer with deionized or distilled water. Put unused solution back at 4°C. If crystals have formed in the concentrate, warm it with 40°C water bath and mix it gently until the crystals have completely dissolved. The solution should be cooled to room temperature before use.

Standard Preparation

- Reconstitute the lyophilized Ang II standard by adding 1 ml of Standard/Sample Dilution Buffer to make the 2000 pg/ml standard stock solution. Use within 2 hours after reconstituting.
- Allow solution to sit at room temperature for 10 minutes, then gently vortex to mix completely.
- Prepare 0.6 ml of 1000 pg/ml top standard by adding 0.3 ml of the above stock solution in 0.3 ml of Standard/Sample Dilution Buffer. Perform 2-fold serial dilutions of the top standards to make the standard curve within the range of this assay.
- Suggested standard points are: 2000, 1000, 500, 250, 125, 62.5, 31.2, 0 pg/ml

Sample Preparation

- Samples to be used within 5 days may be stored at 4°C, otherwise samples must be stored at -20°C (≤1 month) or -80°C (≤2 months) to avoid loss of bioactivity and contamination.
- Avoid multiple freeze-thaw cycles.

Serum: Coagulate the serum for 2 hour at room temperature or overnight at 4°C. Centrifuge at approximately 1000×g for 20 min. Collect the supernatant and carry out the assay immediately. Blood collection tubes should be disposable, non-pyrogenic, and endotoxin free.

Plasma: Collect plasma using EDTA-Na2 as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2 - 8°C within 30 minutes of collection. Collect the supernatant and carry out the assay immediately. Avoid hemolysis, high cholesterol samples.

Tissue homogenates: Rinse the tissues with ice-cold PBS (0.01M, pH=7.4) to remove excess hemolysis blood thoroughly. Tissue pieces should be weighed and then minced to small pieces which will be homogenized in PBS (the volume depends on the weight of the tissue. 9 mL PBS would be appropriate for 1 g of tissue. Some protease inhibitor is recommended to be adedd into the PBS). Homogenize with a glass homogenizer on ice. To further break the cells, sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifuged for 5 minutes at 5000×g to retrieve the supernatant. The total protein concentration can be determined by BCA kit and the total protein concentration of each well sample should not exceed 0.3 mg/ml.

Cell culture supernatant: Centrifuge supernatant for 20 minutes to remove insoluble impurity and cell debris at 1000×g at 2 - 8°C. Collect the clear supernatant and carry out the assay immediately.

Cell culture lysate: Commercial RIPA kits are recommended. Follow the instructions provided. Generally, 0.5 ml RIPA lysis buffer would be appropriate for $2x10^6$ cells, DNA must be removed. The total protein concentration can be determined by BCA kit and the total protein concentration of each well sample should not exceed 0.3 mg/ml.

Other biological fluids: Centrifuge samples for 20 min at 1000×g at 4°C. Collect the supernatant and carry out the assay immediately.

∆ Note: *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.*

Assay Procedure

- Bring all reagents and samples to room temperature 30 minutes prior to the assay.
- It is recommended that all standards and samples be run at least in duplicate.
- A standard curve should be run for each assay.

1. Prepare all reagents, samples, and standards.
2. Wash plate 2 times with 1X Wash Solution before adding standard, sample and control wells.
3. Add 50 µl of each standards or samples into appropriate wells. Immediately add 50 µl of Biotin-detection antibody working solution to each well and cover the plate with plate sealer.
4. Gently tap the plate and incubate at 37°C for 45 min.
5. Discard the solution and wash 3 times with 1X Wash Solution. Wash by filling each well with Wash Buffer (350 µl) using a multi-channel pipette or autowasher. Let it soak for 1-2 minutes, and then remove all residual wash-liquid from the wells by aspiration. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Clap the plate on absorbent filter papers or other absorbent materials.
6. Add 0.1 ml of SABC working solution into each well, cover the plate and incubate at 37°C for 30 min.
7. Discard the solution and wash 5 times with 1X Wash Solution as step 5.
8. Add 90 µl of TMB substrate into each well, cover the plate and incubate at 37 °C in dark within 15-30 min. The shades of blue should be seen in the first 3-4 wells by the end of incubation.
9. Add 50 µl of Stop Solution to each well. Read result at 450 nm within 20 minutes.

Calculations

For calculation, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of Zero well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The Human Ang II concentration of the samples can be interpolated from the standard curve. If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

Recovery:

Matrix	Recovery Range (%)	Average (%)
Serum (n=5)	85-104	95
EDTA Plasma (n=5)	88-102	94
Heparin Plasma (n=5)	85-101	92

Linearity:

Sample	1:2	1:4	1:8
Serum (n=5)	85-101%	87-105%	90-104%
EDTA Plasma (n=5)	87-98%	83-98%	84-97%
Heparin Plasma (n=5)	80-91%	85-100%	80-100%

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips: **www.abcam.com/protocols/the-complete-elisa-guide**

For technical support contact information, visit: www.abcam.com/contactus

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