AGT (Human/Mouse/Rat) ELISA Kit

Catalog Number KA1679

96 assays

Version: 06

Intended for research use only

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Introduction

Intended Use

The AGT (Human/Mouse/Rat) ELISA Kit is an in vitro quantitative assay for detecting Angiotensin II peptide based on the principle of Competitive Enzyme Immunoassay.

Background

Angiotensin, a key player in the renin-angiotensin system, is a peptide hormone that causes vasoconstriction, increased blood pressure, and release of aldosterone from the adrenal cortex. It is derived from the precursor molecule angiotensinogen produced in the liver.

Angiotensin II is formed from Angiotensin I, which is removed of two terminal residues by the enzyme Angiotensin-converting enzyme (ACE). Angiotensin II acts as an endocrine, autocrine/ paracrine, and intracrine hormone. Angiotensin II is degraded to angiotensin III by angiotensinases that are located in red blood cells and the vascular beds of most tissues. It has a half-life in circulation of around 30 seconds, while in tissue, it may be as long as 15-30 minutes.

The effect of obesity on Angiotensin II has recently been reported. Obese patients show heightened renal vasodilation to blockade of the reninangiotensin system, suggesting deficits in vascular responses to angiotensin II. This may due to increases reactivity of renal vasoconstriction to ANG II.

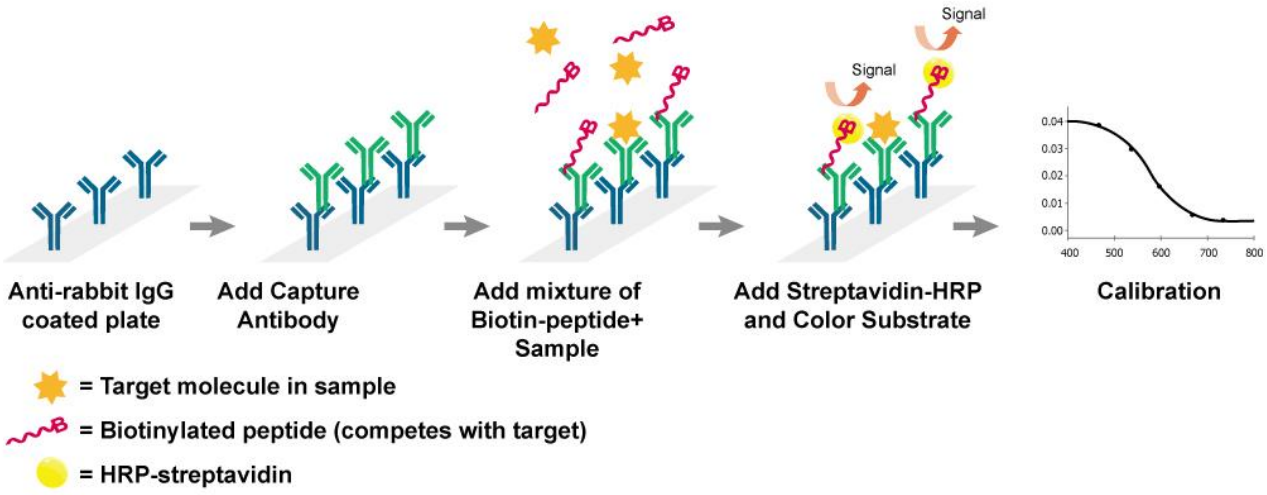
Angiotensin II has been associated with a number of important physiological processes in heart, brain, adrenal gland and kidney. For cardiovascular effect, Angiotensin II is a potent direct vasoconstrictor, constricting arteries and veins and increasing blood pressure. It is also the most important Gq stimulator of the heart during hypertrophy. For neural effects, Angiotensin II increases thirst sensation (dipsogen) through the subfornical organ (SFO) of the brain, decreases the response of the baroreceptor reflex, and increases the desire for salt. It increases secretion of ADH in the posterior pituitary and secretion of ACTH in the anterior pituitary. For adrenal effects, Angiotensin II acts on the adrenal cortex, causing it to release aldosterone. For renal effects, Angiotensin II has a direct effect on the proximal tubules to increase Na^+ absorption.

Principle of the Assay

The microplate in the kit is pre-coated with anti-rabbit secondary antibody. After a blocking step and incubation of the plate with anti-Angiotensin II antibody, both biotinylated Angiotensin II peptide and peptide standard or targeted peptide in samples interacts competitively with the Angiotensin II antibody. Uncompeted (bound) biotinylated Angiotensin II peptide then interacts with Streptavidin-horseradish peroxidase (SA-HRP) which catalyzes a color development reaction. The intensity of colorimetric signal is directly proportional to the amount of biotinylated peptide-SA-HRP complex and inversely proportional to the amount of Angiotensin II

peptide in the standard or samples. This is due to the competitive binding to Angiotensin II antibody between biotinylated Angiotensin II peptide and peptides in standard or samples. A standard curve of known concentration of Angiotensin II peptide can be established and the concentration of Angiotensin II pe ptide in the samples can be calculated accordingly.

This kit can theoretically detect all active angiotensins, including ANGI, ANGII, ANGIII and ANGIV. However, it does not detect inactive angiotensinogen.



General Information

Materials Supplied

List of component

Component	Amount
Angiotensin II Microplate (Item A): 96 wells (12 strips x 8 wells) coated with secondary antibody.	96 (8x12) wells
Wash Buffer Concentrate (20x) (Item B)	25 ml
Lyophilized standard Angiotensin II Peptide (Item C)	2 vials
Lyophilized anti-Angiotensin II polyclonal antibody (Item N)	2 vials
1x Assay Diluent E (Item R): Diluent for both standards and samples including serum, plasma, cell culture media or other sample types.	25 ml x 2
Lyophilized biotinylated Angiotensin II peptide (Item F)	2 vials
HRP-Streptavidin concentrate (Item G): 200x concentrated HRP-conjugated Streptavidin	600 µl
Lyophilized positive control (Item M)	1 vial
TMB One-Step Substrate Reagent (Item H): 3, 3', 5, 5'- tetramethylbenzidine (TMB) in buffered solution.	12 ml
Stop Solution (Item I): 0.2 M sulfuric acid.	8 ml

Storage Instruction

- ✓ Standard, Biotinylated Angiotensin II peptide, and Positive Control should be stored at -20°C after arrival. Avoid multiple freeze-thaws.
- ✓ The remaining kit components may be stored at 4°C.
- ✓ Opened Microplate Wells and antibody (Item N) may be stored for up to 1 month at 2° to 8°C. Return unused wells to the pouch containing desiccant pack, reseal along entire edge.

Materials Required but Not Supplied

- ✓ Microplate reader capable of measuring absorbance at 450 nm.
- ✓ Precision pipettes to deliver 2 µl to 1 ml volumes.
- ✓ Adjustable 1-25 ml pipettes for reagent preparation.
- ✓ 100 ml and 1 liter graduated cylinders.
- ✓ Absorbent paper.
- ✓ Distilled or deionized water.
- ✓ SigmaPlot software (or other software which can perform fourparameter logistic regression models)
- ✓ Tubes to prepare standard or sample dilutions.
- ✓ Orbital shaker
- ✓ Aluminum foil
- ✓ Saran Wrap

Assay Protocol

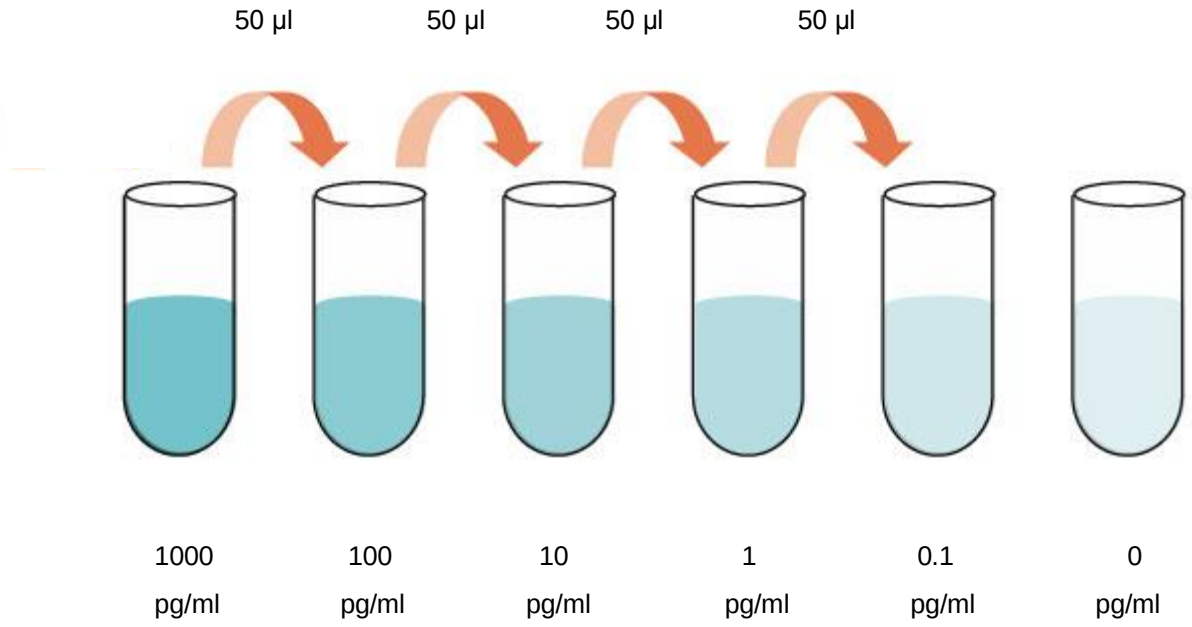
Reagent Preparation

For sample and positive control dilutions, refer to steps 5, 6, 7 and 9 of Reagent Preparation.

1. Keep kit reagents on ice during reagent preparation steps. Equilibrate plate to room temperature before opening the sealed pouch.
2. Briefly centrifuge the Anti-Angiotensin II Antibody vial (Item N) and reconstitute with 5 µl of ddH_2O before use. Add 50 µl of 1x Assay Diluent E into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently.
3. The antibody concentrate should then be diluted 100-fold with 1x Assay Diluent E. This is your anti-Angiotensin II antibody working solution, which will be used in step 2 of the Assay Procedure.

NOTE: the following steps may be done during the antibody incubation procedure (step 2 of Assay Procedure).

4. Briefly centrifuge the vial of biotinylated Angiotensin II peptide (Item F) and reconstitute with 20 µl of ddH_2O before use. Add 10 µl of Item F to 5 ml of the 1X Assay Diluent E. Pipette up and down to mix gently. The final concentration of biotinylated Angiotensin II will be 20 pg/ml. This solution will only be used as the diluent in step 5 of Reagent Preparation.
5. Preparation of Standards: Label 6 microtubes with the following concentrations: 1000 pg/ml, 100 pg/ml, 10 pg/ml, 1 pg/ml, 0.1 pg/ml and 0 pg/ml. Pipette 450 µl of biotinylated Angiotensin II solution into each tube, except for the 1000 pg/ml (leave this one empty). It is very important to make sure the concentration of biotinylated Angiotensin II is 20 pg/ml in all standards.
    a. Briefly centrifuge the vial of standard Angiotensin II peptide (Item C) and reconstitute with 10 µl of ddH_2O. In the tube labeled 1000 pg/ml, pipette 8 µl of Item C and 792 µl of 20 pg/ml biotinylated Angiotensin II solution (prepared in step 4 above). This is your Angiotensin II stock solution (1000 pg/ml Angiotensin II, 20 pg/ml biotinylated Angiotensin II). Mix thoroughly. This solution serves as the first standard.
    b. To make the 100 pg/ml standard, pipette 50 µl of Angiotensin II stock solution the tube labeled 100 pg/ml. Mix thoroughly.
    c. Repeat this step with each successive concentration, preparing a dilution series as shown in the illustration below. Each time, use 450 µl of biotinylated Angiotensin II and 50 µl of the prior concentration until 0.1 pg/ml is reached. Mix each tube thoroughly before the next transfer.
    d. The final tube (0 pg/ml Angiotensin II, 20 pg/ml biotinylated Angiotensin II) serves as the zero standard (or total binding).



6. Prepare a 10-fold dilution of Item F. To do this, add 2 µl of Item F to 18 µl of the 1X Assay Diluent E. This solution will be used in steps 7 and 9.
7. Positive Control Preparation: Briefly centrifuge the positive control vial and reconstitute with 100 µl of ddH_2O before use (Item M). To the tube of Item M, add 101 µl 1x Assay Diluent E. Also add 4 µl of 10-fold diluted Item F (prepared in step 6) to the tube. This is a 2-fold dilution of the positive control. Mix thoroughly. The positive control is a cell culture medium sample with an expected signal between 10% and 30% of total binding (70-90% competition) if diluted as described above. It may be diluted further if desired, but be sure the final concentration of biotinylated Angiotensin II is 20 pg/ml.
8. If Item B (20X Wash Concentrate) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1X Wash Buffer.
9. Sample Preparation: Use 1X Assay Diluent E + biotinylated ANG II to dilute samples, including serum/plasma, cell culture medium and other sample types. It is very important to make sure the final concentration of the biotinylated Angiotensin II is 20 pg/ml in every sample. EXAMPLE: to make a 4-fold dilution of sample, mix together 5 µl of 10-fold diluted Item F (prepared in step 6), 182.5 µl of 1X Assay Diluent E, and 62.5 µl of your sample; mix gently. The total volume is 250 µl, enough for duplicate wells on the microplate.

   Do not use Item F diluent from Step 5 for sample preparation.

   If you plan to use undiluted samples, you must still add biotinylated Angiotensin II to a final concentration of 20 pg/ml. EXAMPLE: Add 5 µl of 10-fold diluted Item F to 245 µl of sample. *NOTE: Optimal sample dilution factors should be determined empirically.*
10. Briefly centrifuge the HRP-Streptavidin vial (Item G) before use. The HRP-Streptavidin concentrate should be diluted 200-fold with 1X Assay Diluent E.

Assay Procedure

1. Keep kit reagents on ice during reagent preparation steps. It is recommended that all standards and samples be run at least in duplicate.
2. Add 100 µl anti-Angiotensin II antibody (see Reagent Preparation step 3) to each well. Incubate for 1.5 hours at room temperature with gentle shaking (1-2 cycles/sec). You may also incubate overnight at 4°C.
3. Discard the solution and wash wells 4 times with 1x Wash Buffer (200-300 µl each). Washing may be done with a multichannel pipette or an automated plate washer. Complete removal of liquid at each step is essential to good assay performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
4. Add 100 µl of each standard (see Reagent Preparation step 5), positive control (see Reagent Preparation step 7) and sample (see Reagent Preparation step 9) into appropriate wells. Be sure to include a blank well (Assay Diluent only). Cover wells and incubate for 2.5 hours at room temperature with gentle shaking (1-2 cycles/sec) or overnight at 4°C.
5. Discard the solution and wash 4 times as directed in Step 3.
6. Add 100 µl of prepared HRP-Streptavidin solution (see Reagent Preparation step 10) to each well. Incubate for 45 minutes at room temperature. It is recommended that incubation time should not be shorter or longer than 45 minutes.
7. Discard the solution and wash 4 times as directed in Step 3.
8. Add 100 µl of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking (1-2 cycles/sec).
9. Add 50 µl of Stop Solution (Item I) to each well. Read absorbances at 450 nm immediately.

Summary

1. Prepare all reagents, samples and standards as instructed.
2. Add 100 µl anti-Angiotensin II antibody to each well. Incubate 1.5 hours at room temperature or overnight at 4°C.
3. Add 100 µl standard or sample to each well. Incubate 2.5 hours at room temperature or overnight at 4°C.
4. Add 100 µl prepared Streptavidin solution. Incubate 45 minutes at room temperature.
5. Add 100 µl TMB One-Step Substrate Reagent to each well. Incubate 30 minutes at room temperature.
6. Add 50 µl Stop Solution to each well. Read at 450 nm immediately

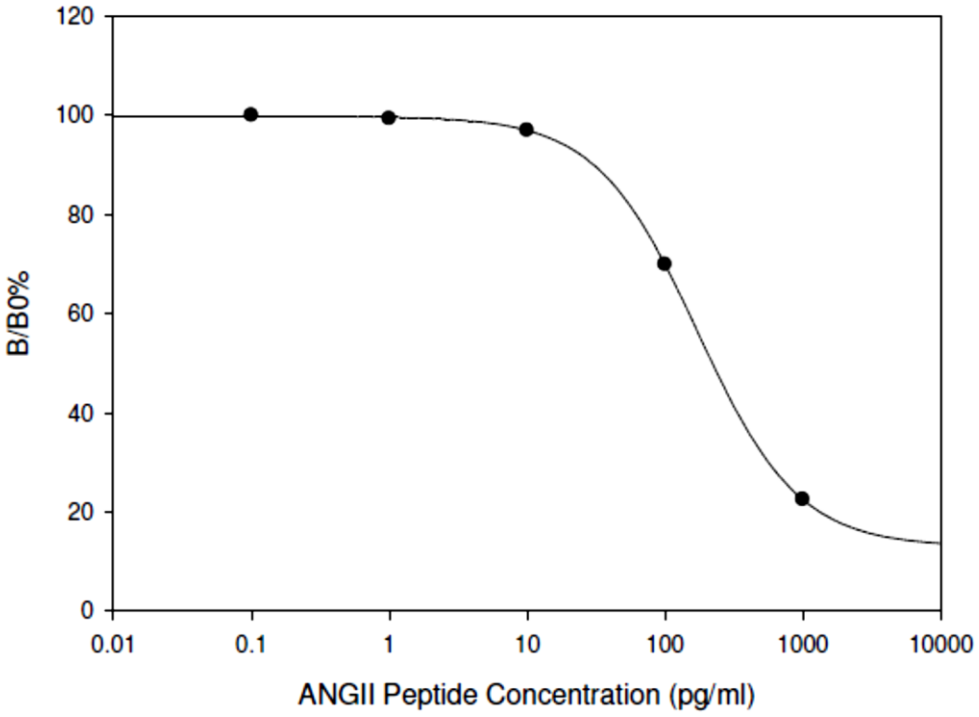
Data Analysis

Calculation of Results

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the blank optical density. Plot the standard curve using SigmaPlot software (or other software which can perform four-parameter logistic regression models), with standard concentration on the x-axis and percentage of absorbance (see calculation below) on the y-axis. Draw the best-fit straight line through the standard points.

Percentage absorbance = (B – blank OD)/(B_0 – blank OD) where
B = OD of sample or standard and
B_0 = OD of zero standard (total binding)

These standard curves are for demonstration only. A standard curve must be run with each assay.



Performance Characteristics

- Sensitivity
  The minimum detectable concentration of Angiotensin II is 21.19 pg/ml.

- Detection Range
  0.1-1,000 pg/ml

- Precision
  Intra-Assay: CV<10%
  Inter-Assay: CV<15%

- Specificity
  Cross Reactivity: This ELISA kit shows no cross-reactivity with any of the cytokines tested: Ghrelin, Nesfatin, NPY and APC.

Resources

Troubleshooting

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standard dilution	Ensure briefly spin the vial of Item C and dissolve the powder thoroughly by a gentle mix.
Low signal	Too brief incubation times	Ensure sufficient incubation time; assay procdure step 2 change to over night
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Large CV	Inaccurate pipetting	Check pipettes
High background	Plate is insufficiently washed	Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed.
	Contaminated wash buffer	Make fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store your standard at ≦-20°C after receipt of the kit.
	Stop solution	Stop solution should be added to each well before measure

References

Skurk T, Lee YM, Hauner H (May 2001). "Angiotensin II and its metabolites stimulate PAI-1 protein release from human adipocytes in primary culture". Hypertension 37 (5): 1336–40.

Plate Layout

	A	B	C	D	E	F	G	H
12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
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