ab136934 – Angiotensin I ELISA Kit

Instructions for Used

For quantitative detection of Angiotensin I in plasma and serum.

This product is for research use only and is not intended for diagnostic use.

Version 6b Last Updated 13 January 2025

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1. BACKGROUND

Abcam's Angiotensin I *in vitro* competitive ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the accurate quantitative measurement of Angiotensin I in plasma and serum.

A goat anti-rabbit IgG antibody has been precoated onto 96-well plates. Standards or test samples are added to the wells, along with a solution of LVV Hemorphin 7 conjugated to biotin, followed by a solution of polyclonal antibody to LVV Hemorphin 7. The plate is washed to remove unbound reagents. A solution of streptavidin-HRP conjugate is then added. After further incubation the excess reagents are washed away and TMB substrate is added, which is catalyzed by HRP to generate a yellow color. A stop solution changes this color from yellow to blue, and the intensity of this blue coloration is inversely proportional to the amount of LVV Hemorphin 7 captured in the plate.

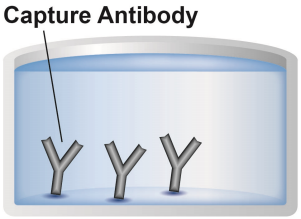
Angiotensins are small peptides derived from angiotensinogen. Several of the known Angiotensins are established endocrine effectors in the regulation of blood pressure, but they are also known to have other functions locally, in several organs and tissues (paracrine) and at the cellular level (autocrine / intracrine). Angiotensin I has no known effector function but it is an immediate precursor of Angiotensin II. Angiotensin II binds AT1 receptors, which promote vasoconstriction, sodium retention, release of aldosterone, release of Arg-vasopressin, cell proliferation, inflammation, fibrosis, anxiety, and cardiac hypertrophy. Angiotensin A and Angiotensin III, also bind AT1 receptors. Angiotensin (1-7) binds a different receptor called MAS-1 which has opposite effects (vasodilation, natriuresis, antiproliferation, NO release, PGE release, and apoptosis). Angiotensin IV binds yet another receptor called AT4 (IRAP), which promotes increase of blood flow, angiogenesis, and natriuresis, and which has also been implicated in memory formation and in the pathogenesis of Alzheimer's disease. The peptide LVV-hemorphin-7, which is not an angiotensin, also binds the AT4 receptor. There are other angiotensin peptides that

have been identified, including Ang (1-9), Ang (1-12), Ang V(3-7), as well as several other shorter peptides that have undetermined functions.

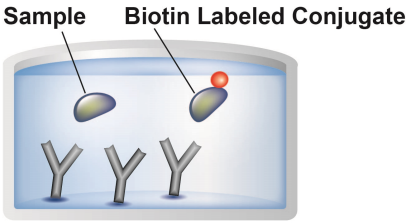
Angiotensins can be present in very low concentrations in some biological samples. In such cases, dilution of samples to avoid “non-specific” interference by any present factors is not productive because the angiotensin analyte is also diluted to levels far below the minimum detection concentration. Thus, investigators have used several procedures for extracting Angiotensins from biological samples prior to using them for immunoassays.

Angiotensins share common sequences, and in some cases they cannot be discriminated by immunoassays. Prior extraction and separation by HPLC may, thus, be required.

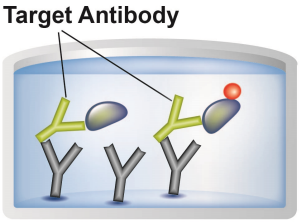
2. ASSAY SUMMARY



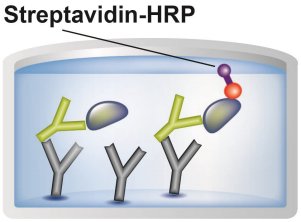
Prepare all reagents and samples as instructed.



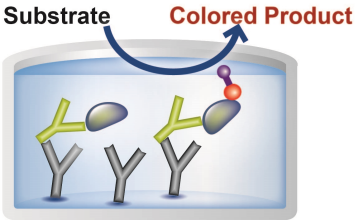
Add samples and Biotinylated antigen and to appropriate wells. Incubate at room temperature.



Add Angiotensin I Polyclonal Antibody to appropriate wells. Incubate at room temperature.



Wash and add prepared labeled Streptavidin HRP-conjugate to appropriate wells. Incubate at room temperature.



Wash and add TMB substrate to each well. Incubate at room temperature. Add Stop Solution to each well. Read immediately.

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

- Stop Solution is a solution of trisodium phosphate. This solution is caustic; care should be taken in use
- We test this kit's performance with a variety of samples, however it is possible that high levels of interfering substances may cause variation in assay results

4. STORAGE AND STABILITY

Store all components at +4°C immediately upon receipt, apart from the Standard and the Conjugate, which should be stored at -20°C. Avoid multiple freeze-thaw cycles.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
Goat anti-Rabbit IgG Microplate (12 x 8 wells)	96 Wells	+4ºC
Angiotensin I Biotinylated Conjugate	4 mL	-20ºC
Angiotensin I Polyclonal Antibody	4 mL	+4ºC
Angiotensin I Standard	1 µg	-20ºC
Assay Buffer 16	30 mL	+4ºC
20X Wash Buffer Concentrate	27 mL	+4ºC
TMB Substrate	10 mL	+4ºC
Stop Solution 2	10 mL	+4ºC
Streptavidin-HRP	12.5 µg	+4ºC

6. MATERIALS REQUIRED, NOT SUPPLIED

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

- Standard microplate reader - capable of reading at 405 nm, preferably with correction between 570 and 590 nm.
- Automated plate washer (optional)
- Adjustable pipettes and pipette tips. Multichannel pipettes are recommended when large sample sets are being analyzed
- Eppendorf tubes
- Microplate Shaker
- Absorbent paper for blotting
- Deionized water
- Phenylmethylsulfonyl fluoride (PMSF)
- Protease inhibitor cocktail (PIC)
- Materials and reagents to extract the analyte from biological fluids or tissues, if needed (see section 11)

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted

8. TECHNICAL HINTS

- Standards can be made up in either glass or plastic tubes
- Pre-rinse the pipette tip with the reagent, use fresh pipette tips for each sample, standard and reagent
- Pipette standards and samples to the bottom of the wells
- Add the reagents to the side of the well to avoid contamination
- This kit uses break-apart microtiter strips, which allow the user to measure as many samples as desired. Unused wells must be kept desiccated at 4°C in the sealed bag provided. The wells should be used in the frame provided
- Care must be taken to minimize contamination by endogenous alkaline phosphatase. Contaminating alkaline phosphatase activity, especially in the substrate solution, may lead to high blanks. Care should be taken not to touch pipet tips and other items that are used in the assay with bare hands
- Prior to addition of substrate, ensure that there is no residual wash buffer in the wells. Any remaining wash buffer may cause variation in assay results
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions**

9. REAGENT PREPARATION

Equilibrate all reagents and samples to room temperature (18 - 25°C) prior to use.

9.1 1X Wash Buffer

Prepare the 1X Wash Buffer by diluting 5 mL of the 20X Wash Buffer Concentrate in 95 mL of deionized water. Mix thoroughly and gently.

9.2 1X Streptavidin-HRP

Reconstitute one vial of Streptavidin-HRP with 250 µL of distilled water and vortex thoroughly. Store at 4ºC for up to 3 months. For prolonged storage, aliquot and freeze at -20°C. Avoid repeated freeze/thaw cycles. Prepare the 1X Streptavidin-HRP by diluting the reconstituted stock 1:1000 in Assay Buffer 16. Example, for 96 wells add 20 µL of reconstituted Streptavidin-HRP to 20 mL of Assay Buffer 16. Mix thoroughly and gently. Do not store diluted Streptavidin-HRP.

10. STANDARD PREPARATIONS

Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards for every use. Reconstitution of the Angiotensin I standard should be prepared no more than 1 hour prior to the experiment. Diluted standards should be used within 60 minutes of preparation.

10.1 Reconstitute one vial of Angiotensin I standard with 1 mL of the assay buffer. Vortex to ensure the entire standard is dissolved.

10.2 Label six tubes with numbers #1 – #6.

10.3 Add 990 µL appropriate diluent (Assay Buffer or Tissue Culture Media) to tube 1

10.4 Add 900 µL appropriate diluent to tube #2

10.5 Add 750 µL appropriate diluent to tubes #3 - #6

10.6 Prepare a 10,000 pg/mL **Standard 1** by adding 10 µL of the 1 µg/mL Stock Standard to tube #1. Vortex thoroughly.

10.7 Prepare **Standard 2** by transferring 100 µL from Standard 1 to tube #2. Vortex thoroughly.

10.8 Prepare **Standard 3** by transferring 250 µL from Standard 2 to tube #3. Vortex thoroughly.

10.9 Using the table below as a guide, repeat for tubes #4 through #6.

Standard	Sample to Dilute	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	Stock	10	990	10,0000	10,000
2	Standard 1	100	900	10,000	1,000
3	Standard 2	250	750	1,000	2500
4	Standard 3	250	750	250	62.5
5	Standard 4	250	750	62.5	15.6
6	Standard 5	250	750	15.6	3.9

11. SAMPLE COLLECTION AND STORAGE

- The assay is suitable for the measurement of Angiotensin I in serum and plasma. Other matrices such as urine and tissue may be suitable, but have not been validated. This kit is not species specific. However, samples containing rabbit IgG will interfere in the assay due to the GxR IgG coated plate. Prior to assay, frozen samples should be brought to 4°C and centrifuged, if necessary, to isolate residual debris
- Please note that recovery of peptides from extraction processes can be variable. It is important to optimize any process to obtain optimum recoveries. Extraction efficiencies can be determined by a number of methods, including the use of radioactive peptide, or by spiking into paired samples and determining the recovery of this known amount of added analyte. The protocol provided below is a suggestion and should not be considered an optimized protocol for any specific analyte.

Suggested Extraction Protocol:

Materials

- 100 or 200 mg C18 columns
- Solutions
- Column activation: Methanol
- Column washing and binding: 1% Trifluoroacetic acid (TFA), 99% Water
- Elution: 60% Acetonitrile (ACN), 1% TFA, 39% water
- Vacuum manifold with Luer syringe stopcocks to control the flow rate through the extraction columns. Control the flow rate (~1ml/min) by opening/closing empty stopcocks or by adjusting the vacuum seal.
- Vacuum line
- 1.5mL polypropylene microfuge tubes
- Microfuge at 4°C

- 15mL screw cap polypropylene tubes
- Floor or table top swinging–bucket centrifuge with proper rotor for 15mL tubes
- Lyophilizer (or a vacuum chamber, a dry-Ice ethanol trap and a powerful vacuum pump)
- Additional standard (excess provided) to determine extraction efficiency

Protocol for 100mg columns. Adjust volumes accordingly if using 200mg columns.

11.1 Prepare the samples

11.1.1 In a microfuge tube add 500µL 1% TFA to 500µL plasma sample.

11.1.2 Mix and centrifuge at maximum speed, cold (4°C), for 20 minutes.

11.1.3 Carefully remove the supernatant and store on ice until C18 column is ready

11.2 Prepare the column

11.2.1 Wash column with 0.5 ml Methanol

11.2.2 Wash column 3 times with 1 mL 1 % TFA (use vacuum manifold, see above)

11.3 Binding

11.3.1 Load prepared sample (1 mL) and let it flow through the column slowly (~ 2 minutes) (use vacuum manifold)

11.3.2 Wash column 2 times with 1 mL 1 % TFA (use vacuum manifold)

11.4 Elution- Up to now all washes were discarded into the vacuum manifold. Now remove the columns from the vacuum manifold.

11.4.1 Place columns on top of a labeled 15 mL polypropylene tube. The top column rims should

prevent the columns from falling into the collection tubes.

11.4.2 Add 1 mL 60% ACN 1% TFA and spin very slowly (300 – 500 rpm) ideally it should take several minutes to elute.

11.5 Lyophilization

11.5.1 Cover the tubes with parafilm and punch small holes with a needle.

11.5.2 Freeze in dry-ice and lyophilize overnight.

11.6 Reconstitution

11.6.1 Add 500µL the assay buffer to re-suspend the pellet at 1 X concentration and assay immediately.

12. PLATE PREPARATION

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents
- Unused well strips should be returned to the plate packet and stored at 4°C
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates)
- Well effects have not been observed with this assay. Contents of each well can be recorded on the template sheet included in the Resources section

	1	**2**	**3**	**4**
A	B_s	Std 2	Std 6	Sample 4
B	B_s	Std 2	Std 6	Sample 4
C	NSB	Std 3	Sample 1	etc
D	NSB	Std 3	Sample 1	etc
E	B_0	Std 4	Sample 2	
F	B_0	Std 4	Sample 2	
G	Std 1	Std 5	Sample 3	
H	Std 1	Std 5	Sample 3	

Plate layout shows controls, blanks and standards required for each assay. Use additional strips of wells to assay all your samples.

Key:

B_s = Blank; contains substrate only.

NSB = Non-specific binding; contains standard diluent, assay buffer, conjugate and substrate.

B_0 = 0 pg/mL standard; contains standard diluent, conjugate, antibody and substrate

13. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature prior to use**
- **It is recommended to assay all standards, controls and samples in duplicate**
- **Refer to the recommended plate layout in Section 12 before proceeding with the assay**

13.1 Add 75 µL appropriate diluent* into the NSB (non-specific binding) wells. (*Use the same diluent used to prepare standards in section 10, either Assay Buffer or Tissue Culture Media).

13.2 Add 50 µL of the. Appropriate diluent* into the B_0 wells. (*Use the same diluent used to prepare standards in section 10, either Assay Buffer or Tissue Culture Media).

13.3 Add 50 µL of prepared standards and 50 µL diluted samples to appropriate wells.

13.4 Add 25 µL of Angiotensin I Polyclonal Antibody (yellow) into B_0, standard and sample wells, i.e. not B_s, and NSB wells

13.5 Seal the plate. Incubate the plate at room temperature for 1 hour on a plate shaker (~500 rpm).

13.6 Add 25 µL Angiotensin I conjugate (blue) into all wells except B_s wells.

13.7 Seal the plate. Incubate the plate at room temperature for 1 hour on a plate shaker (~500 rpm).

13.8 Empty the contents of the wells and wash by adding 400 µL of 1X Wash Buffer to every well. Repeat the wash 3 more times for a total of 4 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.

13.9 Add 100 µL 1X Streptavidin-HRP Conjugate into each well except B_s wells.

13.10 Seal the plate. Incubate the plate at room temperature for 1 hour on a plate shaker (~500 rpm).

13.11 Wash as described in step 13.9.

13.12 Add 100 µL of the Substrate solution to every well. Incubate at room temperature for 30 minutes without shaking.

13.13 Add 100 µL Stop Solution into each well. The plate should be read immediately.

13.14 Blank the plate reader against the B_s wells, read the O.D. absorbance at 450 nm, preferably with correction between 570 and 590 nm. If the plate reader is not able to be blanked against the blank wells, manually subtract the mean optical density of the blank wells from all readings.

14. CALCULATIONS

14.1 Calculate the average net absorbance measurement (Average Net OD) for each standard and sample by subtracting the average NSB absorbance measurement from the average absorbance measurement (Average OD) for each standard and sample.

Average Net OD = Average Bound OD - Average NSB OD

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

$$\text{Percent Bound} = \frac{\text{Average Net OD}}{\text{Average Net } B_0 \text{ OD}} \times 100$$

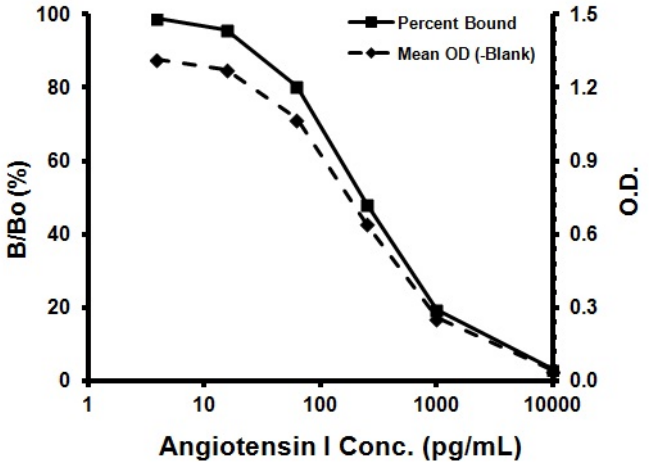
14.3 Plot the Percent Bound (B/B_0) and the net OD versus concentration of Angiotensin I for the standards. The concentration of Angiotensin I in the unknowns can be determined by interpolation of net OD values.

A four parameter algorithm (4PL) provides the best fit, though other equations can be examined to see which provides the most accurate (e.g. linear, semi-log, log/log, 4 parameter logistic). Interpolate protein concentrations for unknown samples from the standard curve plotted.

Samples producing signals greater than that of the highest standard should be further diluted and reanalyzed, then multiplying the concentration found by the appropriate dilution factor

15. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.



Sample	Mean OD ($-B_s$)	% Bound	Angiotensin I pg/mL
B_s	(0.04)	-	-
NSB	0.007	0	-
Standard 1	0.043	3.21	10,000
Standard 2	0.258	19.39	1,000
Standard 3	0.644	48.33	250
Standard 4	1.071	80.41	62.5
Standard 5	1.275	95.78	15.6
Standard 6	1.315	98.77	3.9
B_0	1.332	100	0

16. TYPICAL SAMPLE VALUES

SENSITIVITY –

The sensitivity, minimum detectable dose of Angiotensin I using this Abcam ELISA kit was found to be 4.3 pg/mL. This was determined by the average optical density of the 0 pg/mL Standard and comparing to the average optical density for Standard 6. The detection limit was determined as the concentration of Angiotensin I measured at two standard deviations from the zero along the standard curve.

LINEARITY OF DILUTION –

Pooled human plasma was spiked with 500pg/mL of Angiotensin I, serially diluted 1:2, and run in the assay. Unspiked pooled plasma was processed identically as a background control.

SAMPLE RECOVERY –

Angiotensin I was extracted from spiked pooled human plasma, spiked with 50ng/ml Angiotensin I, following the protocol described above. 75µL of the reconstituted eluate was further fractionated by HPLC. 25µL from each fraction was frozen, lyophilized and reconstituted with 100µL PBS. 50 µL of the resuspended fractions were run in the assay.

Maximum inhibition was obtained with the 16 minute (spiked) and 17 minute (unspiked) fractions. AngI elutes at 16-17 minutes, thus, the material detected by the kit in unspiked pooled human plasma either co-elutes or elutes very close to AngI. It is therefore likely to be composed of specific angiotensin-like peptides. Note that AngI itself is not obviously detected by the HPLC (225 nm). Most of the signal is from materials eluted at 12 minutes, which do not interfere with the ELISA assay, and 18 minutes, which interfere by 50%.

Spiked Conc. (pg/mL)	Dilution Factor	Expected Conc. (pg/mL)	Determined Conc. (pg/mL)	Mean % Recovery
50,000	10	5,000	3,286	66.0
50,000	100	500	448	90.0
50,000	1,000	50	38	76.0

PRECISION –

Intra-Assay

	Angiotensin I (pg/mL)	%CV
Low	25.1	10.0
Medium	53.2	7.3
High	163.6	8.3

Inter-Assay

	Angiotensin I (pg/mL)	%CV
Low	24.4	7.6
Medium	48.1	10.0
High	182.2	7.9

17. ASSAY SPECIFICITY

CROSS REACTIVITY –

The cross re-activities for a number of related compounds were determined by diluting the cross reactants in the kit assay buffer at a concentration in the range of 0.1pM to 500nM. These samples were then measured in the assay:

Analyte	% Cross Reactivities in the range of 0.1pM – 500nM
Ang (1-12)	0.025
Ang I	100
Ang (1-9)	0.000
Ang II	0.083
Ang (1-7)	0.000
Ang A	0.182
Ang III	0.000
Ang IV	0.000
LVV-hemorphin 7	0.000
Bradykinin	0.000

18. TROUBLESHOOTING

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standards dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; change to overnight standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Samples give higher value than the highest standard	Starting sample concentration is too high.	Dilute the specimens and repeat the assay
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the kit	Store the all components as directed

19. NOTES

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)