Version 4 Last updated 18 December 2018

ab222868
Human Annexin A1 ELISA Kit

For the quantitative measurement of human Annexin A1 in plasma, serum, cell lysate and cell culture samples.

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

Table of Contents

1. Overview 1
2. Protocol Summary 2
3. Precautions 3
4. Storage and Stability 3
5. Limitations 4
6. Materials Supplied 4
7. Materials Required, Not Supplied 4
8. Technical Hints 5
9. Reagent Preparation 6
10. Standard Preparation 7
11. Sample Preparation 9
12. Assay Procedure 10
13. Calculations 11
14. Typical Data 12
15. Typical Sample Values 13
16. Assay Specificity 14
17. Species Reactivity 15
18. Troubleshooting 16
19. Notes 18

1. Overview

ab222868 Human Annexin A1 ELISA Kit is designed for the quantitative measurement of Annexin A1 in plasma, serum, cell lysate and cell culture samples.

The kit employs a quantitative sandwich enzyme immunoassay technique that measures human Annexin A1 in approximately 4 hours. A polyclonal antibody specific for human Annexin A1 has been pre-coated onto a 96-well microplate with removable strips. Annexin A1 in standards and samples is sandwiched by the immobilized antibody and biotinylated polyclonal antibody specific for Annexin A1, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

Annexin A1, also known as lipocortin I, annexin I, chromobindin-9, and calpactin II, belongs to the annexin family of calcium-dependent phospholipid-binding proteins. The annexin proteins are structurally characterized by a core domain containing a conserved 70 amino acid sequence repeated four or eight times. This core is preceded by a unique N-terminal domain that varies in sequence and length and is thought to be responsible for specifying different annexin functions. Annexin A1 has an apparent relative molecular mass of 40 kDa with 346 amino acids and is preferentially located on the cytosolic face of the plasma membrane. It mimics the effect of steroids, mediates anti-inflammatory activity and is a potent inhibitor of phospholipase A2 activity. Annexin A1 regulates neutrophil extravasation by interacting with the formyl peptide receptor and triggers different signaling pathways.

2. Protocol Summary

Prepare all reagents, samples, and standards as instructed

Add 50 µL standard or sample to appropriate wells. Incubate at room temperature for 2 hours

Add 50 µL Biotinylated Antibody to all wells. Incubate at room temperature for 1 hour

Wash wells. Add 50 µL Streptavidin-Peroxidase Conjugate to all wells. Incubate at room temperature for 30 minutes

Wash wells. Add 50 µL Chromogen Substrate to all wells. Incubate at room temperature for 15 minutes

Add 50 µL Stop Solution and read OD at 450 nm

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handle with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at 4°C immediately upon receipt, apart from the Human Annexin Ai Standard, Streptavidin-Peroxidase Conjugate and Biotinylated Antibody, which should be stored at -20°C. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. 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.

6. Materials Supplied

Item	Quantity	Storage Condition
Anti-Human Annexin A1 Coated Microplate (12 x 8 wells)	96 wells	+4°C
Human Annexin A1 Standard	1 Vial	-20°C
Biotinylated Human Annexin A1	1 Vial	-20°C
10X Diluent N Concentrate	30 mL	+4°C
20X Wash Buffer Concentrate	2 x 30 mL	+4°C
100X Streptavidin-Peroxidase Conjugate	80 µL	-20°C
Chromogen Substrate	8 mL	+4°C
Stop Solution	12 mL	+4°C
Sealing Tapes	3	+4°C
1X Standard Diluent	2 mL	+4°C

7. Materials Required, Not Supplied

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

- Microplate reader capable of measuring absorbance at 450 nm.
- Deionized water.
- Multi- and single-channel pipettes.
- Tubes for standard dilution.

8. Technical Hints

- 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.
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Make sure all buffers and solutions are at room temperature before starting the experiment.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Make sure you have the right type of plate for your detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on before starting the experiment.

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells.
- Prepare only as much reagent as is needed on the day of the experiment.

9.1 1X Diluent N:

If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the 10X Diluent N Concentrate 1:10 with reagent grade water. Store for up to 30 days at +4°C.

9.2 Biotinylated Human Annexin A1:

Spin down the antibody briefly and dilute the desired amount of the antibody 1:70 with 1X Diluent N. The undiluted antibody should be stored at -20°C.

9.3 1X Wash Buffer Concentrate:

If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the 20X Wash Buffer Concentrate with reagent grade water.

9.4 1X Streptavidin-Peroxidase Conjugate:

Spin down the Streptavidin-Peroxidase conjugate briefly and dilute the desired amount of the conjugate 1:100 with 1X Diluent N. Any remaining solution should be frozen at -20°C.

9.5 Anti-Human Annexin A1 coated Microplate (12 x 8 wells):

Ready to use. Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 30 days in a vacuum desiccator.

9.6 Chromagen Substrate:

Ready to use. Store at +4°C.

9.7 Sealing Tapes:

Ready to use. Store at +4°C.

9.8 Stop Solution:

Ready to use. Store at +4°C.

9.9 Standard Diluent:

Ready to use. Store at +4°C.

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.
- The following section describes the preparation of a standard curve for duplicate measurements (recommended).

10.1 Reconstitute the Annexin A1 Stock to generate a 20 ng/mL **Standard #1**.

10.1.1 First consult the Annexin A1 Standard vial to determine the mass of protein in the vial.

10.1.2 Calculate the appropriate volume of 1X Standard Diluent to add when resuspending the Annexin A1 Standard vial to produce a 20 ng/mL Annexin A1 Standard stock by using the following equation:

CS = Starting mass of Annexin A1 Standard stock (see vial label) (ng)

CF = 20 ng/mL Annexin A1 Standard #1 final required concentration

VD = Required volume of 1X Standard Diluent for reconstitution (µL)

Calculate total required volume 1X Standard Diluent for resuspension:

$$(C_S / C_S) * 1{,}000 = V_D$$

Example:

NOTE: This example is for demonstration purposes only. Please remember to check your standard vial for the actual amount of standard provided.

C_S = 16 ng of Annexin A1 Standard in vial

C_F = 20 ng/mL Annexin A1 **Standard #1** final concentration

V_D = Required volume of 1X Diluent N for reconstitution

(16 ng / 20 ng/mL) * 1,000 = 800 µL

10.1.3 First briefly centrifuge the Annexin A1 Standard Vial to collect the contents on the bottom of the tube.

10.1.4 Reconstitute the Annexin A1 Standard vial by adding the appropriate calculated amount VD of 1X Standard Diluent to the vial to generate the 20 ng/mL Annexin A1 **Standard #1.** Mix gently and thoroughly.

10.2 Allow the reconstituted 20 ng/mL Annexin A1 **Standard #1** to sit for 10 minutes with gentle agitation prior to making subsequent dilutions

10.3 Label seven tubes #2 – 8.

10.4 Prepare duplicate or triplicate standard points by serially diluting the standard stock solution (20 ng/mL) 1:2 with 1X Diluent N to produce 10, 5, 2.5, 1.25, 0.625, and 0.313 ng/mL solutions. 1X Diluent N serves as the zero standard (0 ng/mL). Aliquot standard to limit repeated freezing and thawing. Any remaining solution should be frozen at -20°C and used within 2 days. Avoid repeated freeze-thaw cycles.

10.5 Add 120 µL of 1X Diluent N to tube #2 – 8.

10.6 To prepare **Standard #2**, add 120 µL of the **Standard #1** into tube #2 and mix gently.

10.7 To prepare **Standard #3**, add 120 µL of the **Standard #2** into tube #3 and mix gently.

10.8 Using the table below as a guide, prepare subsequent serial dilutions.

Standard #	Volume to dilute (µL)	Volume Diluent N (µL)	Annexin A1 (ng/mL)
1	Step 10.1		20.0
2	120 µL Standard #1	120	10.0
3	120 µL Standard #2	120	5.0
4	120 µL Standard #3	120	2.50
5	120 µL Standard #4	120	1.25
6	120 µL Standard #5	120	0.625
7	120 µL Standard #6	120	0.313
8 (Blank)	N/A	120	0

11. Sample Preparation

11.1 Plasma:

Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x g for 10 minutes. A 2-fold sample dilution is suggested into 1X Diluent N; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA or Heparin can also be used as an anticoagulant).

11.2 Serum:

Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x g for 10 minutes and remove serum. A 2-fold sample dilution is suggested into 1X Diluent N; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

11.3 Cell Culture Supernatants:

Centrifuge cell culture media at 3000 x g for 10 minutes to remove debris. Collect supernatants and assay. Samples can be stored at -20°C or below. Avoid repeated freeze-thaw cycles.

11.4 Cell Lysate:

Rinse cell with cold PBS and then scrape the cell into a tube with 5 mL of cold PBS and 0.5 M EDTA. Centrifuge suspension at 1500 rpm for 10 minutes at 4°C and aspirate supernatant. Resuspend pellet in ice-cold Lysis Buffer (10 mM Tris, pH 8.0, 130 mM NaCl, 1% Triton X-100, protease inhibitor cocktail). or every 1 x 10^6 cells, add approximately 100 µL of ice-cold Lysis Buffer. Incubate on ice for 60 minutes. Centrifuge at 13000 rpm for 30 minutes at 4°C and collect supernatant. Samples can be stored at -80°C. Avoid repeated freeze-thaw cycles.

12. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.
- Prepare all reagents, working standards, and samples as directed in the previous sections.

12.1 Prepare all reagents, working standards, and samples as directed in the previous sections.

12.2 Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.

12.3 Add 50 µL of Human Annexin A1 Standard or sample per well. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition.

12.4 Wash five times with 200 µL of Wash Buffer manually. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a machine, wash six times with 300 µL of Wash Buffer and then invert the plate, decanting the contents; hit 4-5 times on absorbent material to completely remove the liquid.

12.5 Add 50 µL of Biotinylated Human Annexin A1 Antibody to each well and incubate for 1 hour.

12.6 Wash the microplate as described above (Step 12.4).

12.7 Add 50 µL of Streptavidin-Peroxidase Conjugate to each well and incubate for 30 minutes.

12.8 Turn on the microplate reader and set up the program in advance.

12.9 Wash the microplate as described above (Step 12.4).

12.10 Add 50 µL of Chromogen Substrate per well and incubate for 25 minutes or till the optimal blue color density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip.

12.11 Add 50 µL of Stop Solution to each well. The color will change from blue to yellow.

12.12 Read the absorbance on the microplate reader at a wavelength of 450 nm immediately. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only.

Δ **Note:** Some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

13. Calculations

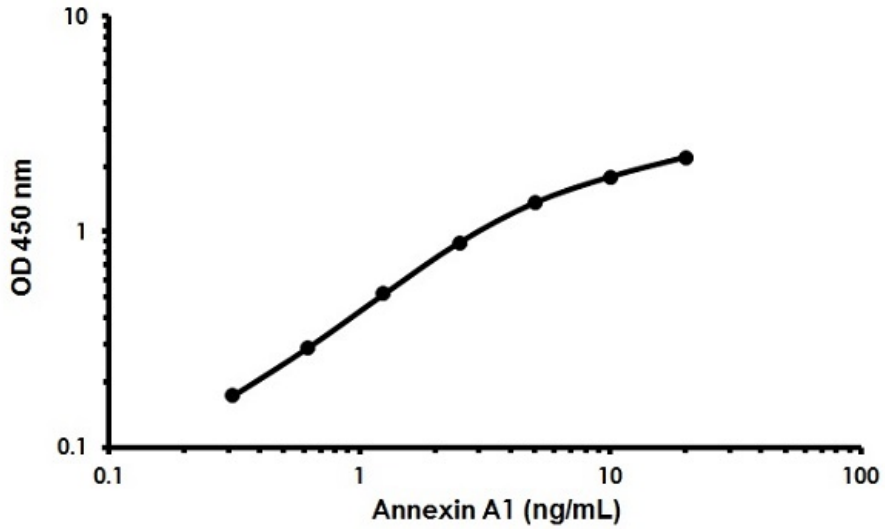
13.1 Calculate the mean value of the duplicate or triplicate readings for each standard and sample.

13.2 To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line can be determined by regression analysis using four-parameter or log-log logistic curve-fit.

13.3 Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only.** A new standard curve must be generated for each assay performed.



Standard Curve Measurements			
Concentration (ng/mL)	O.D 450 nm		Mean O.D
	1	2	
20	2.292	2.138	2.215
10	1.848	1.746	1.797
5	1.399	1.325	1.362
2.5	0.914	0.867	0.891
1.25	0.527	0.501	0.514
0.625	0.296	0.286	0.291
0.313	0.178	0.170	0.174
0	0.080	0.076	0.078
Sample: Pooled Normal, Sodium Citrate Plasma (2x)	0.549	0.435	0.492

Figure 1. Example of human Annexin A1 standard curve.

15. Typical Sample Values

SENSITIVITY –

The minimum detectable dose of Annexin A1 as calculated by 2SD from the mean of a zero standard was established to be 0.13 ng/mL.

PRECISION –

Intra-assay precision was determined by testing replicates of three plasma samples in one assay.

Inter-assay precision was determined by testing three plasma samples in twenty assays.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
CV (%) Recovery %	5.1	5.1	5.3	10.3	10.0	10.4
Average CV (%)	5.2			10.2		

RECOVERY –

Standard Added Value	1.2– 10 ng/mL
Recovery %	92 – 113%
Average Recovery %	97%

Linearity of Dilution

Plasma and serum samples were serially-diluted to test for linearity.

Average Percentage of Expected Value (%)		
Sample Dilution	Plasma	Serum
No Dilution	92%	93%
2	98%	97%
4	105%	102%

16. Assay Specificity

This kit recognizes human Annexin A1 in plasma, serum and cell culture samples.

CROSS REACTIVITY

Proteins	Cross Reactivity (%)
Annexin A1	100%
Annexin A2	None
Annexin A3	None
Annexin A4	None
Annexin A5	None
Annexin A10	None
Annexin A13	None

No significant cross-reactivity observed when protein was tested at 50 ng/mL.

17. Species Reactivity

This kit recognizes human Annexin A1.

Species	Cross Reactivity (%)
Bovine	None
Canine	None
Monkey	60
Mouse	20
Rat	70
Swine	40
Rabbit	None
Human	100

REFERENCE VALUE -

Human plasma and serum samples from healthy adults were tested (n=30). On average, annexin A1 level was 2.5 ng/mL.

Please contact our Technical Support team for more information.

18. Troubleshooting

Problem	Reason	Solution
Low Precision	Use of expired components	Check the expiration date listed before use. Do not interchange components from different lots.
	Improper wash step	Check that the correct wash buffer is being used. Check that all wells are dry after aspiration. Check that the microplate washer is dispensing properly. If washing by pipette, check for proper pipetting technique.
	Splashing of reagents while loading wells	Pipette properly in a controlled and careful manner.
	Inconsistent volumes loaded into wells	Pipette properly in a controlled and careful manner. Check pipette calibration. Check pipette for proper performance.
	Insufficient mixing of reagent dilutions	Thoroughly agitate the lyophilized components after reconstitution. Thoroughly mix dilutions.
	Improperly sealed microplate	Check the microplate pouch for proper sealing. Check that the microplate pouch has no punctures. Check that three desiccants are inside the microplate pouch prior to sealing

Unexpectedly Low or High Signal Intensity	Microplate was left unattended between steps	Each step of the procedure should be performed uninterrupted.
	Omission of step	Consult the provided procedure for complete list of steps.
	Steps performed in incorrect order	Consult the provided procedure for the correct order.
	Insufficient amount of reagents added to wells	Check pipette calibration. Check pipette for proper performance.
	Wash step was skipped	Consult the provided procedure for all wash steps.
	Improper wash buffer	Check that the correct wash buffer is being used.
	Improper reagent preparation	Consult reagent preparation section for the correct dilutions of all reagents.
	Insufficient or prolonged incubation periods	Consult the provided procedure for correct incubation time.
Deficient Standard Curve fit	Non-optimal sample dilution	Sandwich ELISA: If samples generate OD values higher than the highest standard point (P1), dilute samples further and repeat the assay. User should determine the optimal dilution factor for samples.
	Contamination of reagents.	A new tip must be used for each addition of different samples or reagents during the assay procedure.
	Contents of wells evaporate	Verify that the sealing film is firmly in place before placing the assay in the incubator or at room temperature.
	Improper pipetting	Pipette properly in a controlled and careful manner. Check pipette calibration. Check pipette for proper performance.
	Insufficient mixing of reagent dilutions	Thoroughly agitate the lyophilized components after reconstitution. Thoroughly mix dilutions.

19. Notes

Technical Support

Austria
wissenschaftlicherdienst@abcam.com | 019-288-259
France
supportscientifique@abcam.com | 01.46.94.62.96
Germany
wissenschaftlicherdienst@abcam.com | 030-896-779-154
Spain
soportecientifico@abcam.com | 91-114-65-60

Switzerland
technical@abcam.com
Deutsch: 043-501-64-24 | Français: 061-500-05-30
UK, EU and ROW
technical@abcam.com | +44(0)1223-696000

Canada
ca.technical@abcam.com | 877-749-8807
US and Latin America
us.technical@abcam.com | 888-772-2226

Asia Pacific
hk.technical@abcam.com | (852) 2603-6823
China
cn.technical@abcam.com | +86-21-5110-5938 | 400-628-6880
Japan
technical@abcam.co.jp | +81-(0)3-6231-0940
Singapore
sg.technical@abcam.com | 800 188-5244

Australia
au.technical@abcam.com | +61-(0)3-8652-1450
New Zealand
nz.technical@abcam.com | +64-(0)9-909-7829