

ab283976 – Human Annexin A3/ANXA3 ELISA Kit

For the quantitative measurement of A3/ANXA3 in human plasma, serum, urine, tissue, and cell lysate samples.

For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab283976

Storage and Stability: Store kit at +4°C immediately upon receipt, apart from the Standard, SP 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.

Materials Supplied

Item	Quantity	Storage Condition
100X Streptavidin-Peroxidase Conjugate	80 µL	-20°C
10X Diluent M Concentrate	20 mL	4°C
1X Standard Diluent	2 mL	4°C
20X Wash Buffer Concentrate	2 x 30 mL	4°C
Annexin A3/ANXA3 Standard	1 vial	-20°C
Chromogen Substrate	7 mL	4°C
Human Annexin A3/ANXA3 Microplate	96 wells	4°C
Sealing Tapes	3 units	N/A
Stop Solution	11 mL	4°C
50X Biotinylated Human Annexin A3/ANXA3 Antibody	1 vial	-20°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

Pipettes (1-20 µL, 20-200 µL, 200-1000 µL, and multiple channel)

Deionized or distilled reagent grade water

Reagents 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.

If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.

Δ Note: Concentration of the kit components are lot-specific, and the end user should always refer to the vial label.

10X Diluent M Concentrate: Dilute the Diluent M Concentrate 1:10 with reagent grade water to produce a 1x solution. When diluting the concentrate, make sure to rinse the bottle thoroughly to extract any precipitates left in the bottle. Store for up to 30 days at 4°C.

100X Biotinylated Human Annexin A3/ANXA3 Antibody: Spin down the antibody briefly and dilute the desired amount of the antibody 1:100 with Diluent M to produce a 1x solution. The undiluted antibody should be stored at -20°C.

20X Wash Buffer Concentrate: Dilute the Wash Buffer Concentrate 1:20 with reagent grade water to produce a 1x solution. When diluting the concentrate, make sure to rinse the bottle thoroughly to extract any precipitates left in the bottle.

100X Streptavidin-Peroxidase Conjugate: Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with Diluent M to produce a 1x solution. The undiluted conjugate should be stored at -20°C.

Standard Preparation

Always prepare a fresh set of standards for every use.

Prepare serially diluted standards immediately prior to use.

Any remaining standard should be stored at -20°C after reconstitution and used within 30 days. The following section describes the preparation of a standard curve for duplicate measurements (recommended).

Reconstitution of the A3/ANXA3 Standard vial to prepare a 100 ng/ml Stock Standard.

- First consult the A3/ANXA3 Standard vial to determine the mass of protein in the vial.
- Calculate the appropriate volume of 1X Diluent M to add when resuspending the A3/ANXA3 Standard vial to produce a 100 ng/ml A3/ANXA3 Stock Standard by using the following equation:
 - o C_s = Starting mass of A3/ANXA3 Standard (see vial label) (µg)
 - o C_f = The 100 ng/ml A3/ANXA3 Stock Standard final required concentration
 - o V_D = Required volume of 1X Diluent M for reconstitution (µL)
 - o Calculate total required volume 1X Diluent M for resuspension:

$$(C_s / C_f) \times 1,000 = V_D$$

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

C_s = 125 ng of Human Annexin A3/ANXA3 Standard in vial

C_f = 100 ng/mL Human Annexin A3/ANXA3 Standard #1 final concentration

V_D = Required volume of 1X Diluent M for reconstitution (125 ng / 100 ng/mL) x 1,000 = 1250 µL

- Reconstitute the Human Annexin A3/ANXA3 Standard vial by adding the appropriate calculated amount V_D of 1X Diluent M to the vial to generate the 100 ng/mL Human Annexin A3/ANXA3 Standard #1. Mix gently and thoroughly.
- Allow the reconstituted 100 ng/mL Annexin A3/ANXA3 Standard #1 to sit for 10 minutes with gentle agitation prior to making subsequent dilutions
- Label five tubes #2 – 8.
- Add 120 µL of 1X Diluent M to tube #2 – 8.
- To prepare Standard #2, add 120 µL of the Standard #1 into tube #2 and mix gently.
- To prepare Standard #3, add 120 µL of the Standard #2 into tube #3 and mix gently

Using the table below as a guide, prepare subsequent serial dilutions. 1X Diluent M serves as the zero standard (0 ng/mL).

Standard #	Volume to dilute (µL)	Volume 1X Diluent M (µL)	A3/ANXA3 (ng/ml)
1	240 µL	-	100
2	120 µL Standard #1	120	50
3	120 µL Standard #2	120	25
4	120 µL Standard #3	120	12.5
5	120 µL Standard #4	120	6.25
6	120 µL Standard #5	120	3.125
7	120 µL Standard #6	120	1.563
8	-	120	0.0

Sample Preparation

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 and collect plasma.

The sample is suggested for use at 1x; 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).

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.

The sample is suggested for use at 1x; 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.

Urine: Collect urine using sample pot.

Centrifuge samples at 800 x g for 10 minutes.

The sample is suggested for use at 1x or within the range of 2x-5x in Diluent M; 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.

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).

For every 1×10^6 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.

If necessary, dilute samples into Diluent M; user should determine optimal dilution factor depending on application needs.

The undiluted samples can be stored at -80°C.

Avoid repeated freeze-thaw cycles.

Tissue: Extract tissue samples with 0.1 M phosphate-buffered saline (pH 7.4) containing 1% Triton X-100 and centrifuge at 14000 x g for 20 minutes.

Collect the supernatant and measure the protein concentration.

If necessary, dilute samples into Diluent M; user should determine optimal dilution factor depending on application needs. Store remaining extract at -80°C. Avoid repeated freeze-thaw cycles.

Δ Note: Applicable samples may also include biofluids, cell culture, and tissue homogenates. If necessary, user should determine optimal dilution factor depending on application needs.

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 plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4°C.

For each assay performed, a minimum of two wells must be used as the zero control.

For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

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.

1. Add 50 µl of Annexin A3/ANXA3 Standard or sample to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition.
2. Wash the microplate manually or automatically using a microplate washer. Invert the plate and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If washing manually, wash five times with 200 µl of Wash Buffer per well. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a microplate washer, wash six times with 300 µl of Wash Buffer per well; invert the plate and hit 4-5 times on absorbent material to completely remove the liquid.
3. Add 50 µl of Biotinylated Human Annexin A3/ANXA3 Antibody to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 1 hour.
4. Wash the microplate as described above.
5. Add 50 µl of SP Conjugate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
6. Wash the microplate as described above.
7. Add 50 µl of Chromogen Substrate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Incubate in ambient light for 20 minutes or until the optimal blue colour density develops.
8. Add 50 µl of Stop Solution to each well. The colour will change from blue to yellow. Gently tap plate to ensure thorough mixing. Break any bubbles that may have formed.
9. Read the absorbance on a 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. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:

www.abcam.com/protocols/the-complete-elisa-guide

For all technical or commercial enquiries please go to:

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Additional information

CALCULATION

1. Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
2. To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance (OD) on the y-axis. The best fit line can be determined by regression analysis using log-log or four-parameter logistic curve fit.
3. Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

TYPICAL DATA

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

Standard Point	ng/ml	OD	Average OD
1	100	2.078 2.128	2.103
2	50	1.705 1.751	1.728
3	25	1.283 1.251	1.267
4	12.5	0.868 0.842	0.855
5	6.25	0.539 0.521	0.530
6	3.125	0.333 0.345	0.339
7	1.563	0.235 0.233	0.234
8	0.0	0.104 0.098	0.101

PERFORMANCE CHARACTERISTICS

1. The assay recognizes both natural and recombinant human annexin A3.
2. The minimum detectable dose of human annexin A3 as calculated by 2SD from the mean of a zero standard was established to be 0.68 ng/ml.
3. Intra-assay precision was determined by testing three plasma samples twenty times in one assay.
4. Inter-assay precision was determined by testing three plasma samples in twenty assays.

	Intra-Assay Precision	Inter-Assay Precision
CV (%)	5.8	10.5

RECOVERY

Standard Added Value	6.25 – 50 ng/ml
Recovery %	90 – 113
Average Recovery %	97

LINEARITY

Average Percentage of Expected Value (%)		
Sample Dilution	Plasma	Serum
1x	99	101
2x	107	93
4x	94	105

CROSS REACTIVITY

Species	Cross-Reactivity (%)
Canine	None
Bovine	None
Monkey	100%
Mouse	70%
Rat	70%
Swine	80%
Rabbit	None
Protein	Cross-Reactivity (%)
Annexin A4	<3%
Annexin A7	<2%

- No significant cross-reactivity observed with annexin A1, A2, A5, A8, A10, A11, and A13.

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:

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