ab283993 – Human Annexin-A7/ANXA7 ELISA Kit

For the quantitative measurement of Annexin A7 in human cell lysate samples.
For research use only - not intended for diagnostic use.

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.

Materials Supplied

Item	Quantity	Storage Condition
100X Streptavidin-Peroxidase Conjugate	80 µL	-20°C
10X Diluent N Concentrate	30 mL	4°C
1X Standard Diluent	2 mL	4°C
20X Wash Buffer Concentrate	2 x 30 mL	4°C
40X Biotinylated Human Annexin-A7/ANXA7 Antibody	1 vial	-20°C
Annexin-A7/ANXA7 Standard	1 vial	-20°C
Chromogen Substrate	8 mL	-4°C
Human Annexin-A7/ANXA7 Microplate	96 wells	4°C
Sealing Tapes	3 units	4°C
Stop Solution	12 mL	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
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 N Concentrate: Dilute the Diluent N Concentrate 1:10 with reagent grade water to produce a 1x solution. Store for up to 30 days at 2-8°C.

40X Biotinylated Human Annexin A7 Antibody: Spin down the antibody briefly and dilute the desired amount of the antibody 1:40 with Diluent N 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.

100X Streptavidin-Peroxidase Conjugate: Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with Diluent N 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 Annexin-A7 Standard vial to prepare an **80 ng/ml Stock Standard**.

- First consult the Annexin-A7 Standard vial to determine the mass of protein in the vial.
- Calculate the appropriate volume of Standard Diluent to add when resuspending the Annexin-A7 Standard vial to produce an 80 ng/ml Stock Standard by using the following equation:
    - $\mathbf{C_S}$ = Starting mass of Annexin-A7 Standard (see vial label) (ng)
    - $\mathbf{C_F}$ = The 80 ng/ml Annexin-A7 Stock Standard final required concentration
    - $\mathbf{V_D}$ = Required volume of Standard Diluent for reconstitution (µl)
    - Calculate total required volume Standard Diluent for resuspension:

$$\mathbf{(C_S / C_F) \times 1{,}000 = V_D}$$

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

$\mathbf{C_S}$ = 60 ng of Annexin-A7 Standard in vial
$\mathbf{C_F}$ = 80 ng/ml Annexin-A7 Stock Standard final concentration
$\mathbf{V_D}$ = Required volume of Standard Diluent for reconstitution (60 ng / 80 ng/ml) x 1,000 = 750 µl

- Reconstitute the Annexin-A7 Standard vial by adding the appropriate calculated amount VD of Standard Diluent to the vial to generate the 80 ng/ml Annexin-A7 Stock Standard. Mix gently and thoroughly.
- Allow the reconstituted 80 ng/ml Annexin-A7 Stock Standard to sit for 10 minutes with gentle agitation prior to making subsequent dilutions
- Label tubes #1 – 8.
- Add 120 µl of **1X Diluent N** to tubes #1 – 8.
- To prepare Standard #1, add 120 µL of the Stock Standard into tube #1 and mix gently.
- To prepare Standard #2, add 120 µL of the Standard #1 into tube #2 and mix gently
- Using the table below as a guide, prepare subsequent serial dilutions. 1X Diluent N serves as the zero standard (0 ng/mL).

Standard #	Volume to dilute (µL)	Volume 1X Diluent N (µL)	Annexin-A7 (ng/ml)
1	120 (Stock Standard 80 ng/mL)	120	40
2	120 (Standard #1)	120	20
3	120 (Standard #2)	120	10
4	120 (Standard #3)	120	5
5	120 (Standard #4)	120	2.5
6	120 (Standard #5)	120	1.25
7	120 (Standard #6)	120	0.625
8	-	120	0.0

Sample Preparation

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 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.

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 Human Annexin A7 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 A7 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 for 25 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.

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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.

Standard #	ng/mL	OD	Average OD
1	40	2.474 2.350	2.412
2	20	1.637 1.540	1.589
3	10	0.918 0.886	0.902
4	5.0	0.532 0.515	0.523
5	2.5	0.281 0.302	0.292
6	1.25	0.181 0.158	0.170
7	0.625	0.131 0.109	0.120
8	0.0	0.048 0.042	0.045

PERFORMANCE CHARACTERISTICS

1. This assay recognizes both natural and recombinant human annexin A7.
2. The minimum detectable dose of human annexin A7 as calculated by 2SD from the mean of a zero standard was established to be 0.23 ng/ml.
3. Intra-assay precision was determined by testing three reference control samples twenty times in one assay.
4. Inter-assay precision was determined by testing three reference control samples in twenty assays.

	Intra-Assay Precision	Inter-Assay Precision
CV (%)	5.2	8.1

RECOVERY

Standard Added Value	2.5 – 20 ng/ml
Recovery %	93 – 109
Average Recovery %	103

LINEARITY

Average Percentage of Expected Value (%)	
Sample Dilution	A549 (human adenocarcinoma) Cell Culture Lysate
1:10	92
1:20	97
1:40	112

CROSS REACTIVITY

Protein	Cross-Reactivity (%)
Annexin A5	<2

No significant cross-reactivity observed with annexin A1, A3, A4, A8, A10, A11, and A13.

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

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Version 4a | 11 June 2026