AssayMax™

Human Annexin A7 ELISA Kit

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For any questions regarding troubleshooting or performing the assay, please contact our support team at support@assaypro.com.

Thank you for choosing Assaypro.

Assay Summary

Step 1. Add 50 µl of Standard or Sample per well.
Incubate 2 hours.

Step 2. Wash, then add 50 µl of Biotinylated Antibody per well.
Incubate 1 hour.

Step 3. Wash, then add 50 µl of SP Conjugate per well.
Incubate 30 minutes.

Step 4. Wash, then add 50 µl of Chromogen Substrate per well.
Incubate 20 minutes.

Step 5. Add 50 µl of Stop Solution per well.
Read at 450 nm immediately.

Symbol Key

Consult instructions for use.

Assay Template

	A	B	C	D	E	F	G	H
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AssayMax™ Human Annexin A7 (ANXA7) ELISA Kit

Catalog No. EA3607-1

Sample insert for reference use only

Introduction

Annexin A7, also known as synexin, is a part of the group A annexin family. Annexin A7 exists in two isoforms. The first is the 47 kDa isoform found in all tissues with the exception of skeletal muscle. The second isoform is the 51 kDa isoform found in the brain, heart, and skeletal muscle (1-2). In hormone relevant prostate and breast cancers and heptacellular carcinoma (HCC), annexin A7 has proven to function as a tumor suppressor and a possible novel therapeutic target for HCC (2-3). In gastric cancer, one study showed annexin A7 to be an important factor in distant metastasis (2). Malignant glioma, glioblastoma multiform, melanoma, and prostate cancer exhibit decreased levels of annexin A7, while liver cancer, gastric cancer, nasopharyngeal carcinoma, colorectal cancer, cervical squamous cell carcinoma, and breast cancer show increased levels of annexin A7 from normal tissue. Annexin A7 is also being considered as a possible biomarker for the diagnosis, prognosis, and treatment of gastric adenocarcinoma (4).

Principle of the Assay

The AssayMax™ Human Annexin A7 ELISA (Enzyme-Linked Immunosorbent Assay) Kit is designed for detection of annexin A7 in human **cell lysate samples**. This assay employs a quantitative **sandwich enzyme immunoassay** technique that measures human annexin A7 in approximately 4 hours. A polyclonal antibody specific for human annexin A7 has been pre-coated onto a 96-well microplate with removable strips. Annexin A7 in standards and samples is sandwiched by the immobilized antibody and a biotinylated polyclonal antibody specific for human annexin A7, which is recognized by a streptavidin-peroxidase (SP) 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.

Caution and Warning

- This product is for **Research Use Only** and is not intended for use in diagnostic procedures.

- Prepare all reagents (diluent buffer, wash buffer, standard, biotinylated antibody, and SP conjugate) as instructed, prior to running the assay.
- Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this insert. However, the user should determine the optimal dilution factor.
- Spin down the SP conjugate vial, the biotinylated antibody vial, and the standard diluent vial before opening and using contents.
- The Stop Solution is an acidic solution.
- The kit should not be used beyond the expiration date.

Reagents

- **Human Annexin A7 Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human annexin A7.
- **Sealing Tapes:** Each kit contains 3 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **Human Annexin A7 Standard:** Human annexin A7 in a buffered protein base (80 ng, lyophilized).
- **Biotinylated Human Annexin A7 Antibody (50x):** A 50-fold concentrated biotinylated polyclonal antibody against human annexin A7 (120 µl).
- **MIX Diluent Concentrate (10x):** A 10-fold concentrated buffered protein base (30 ml).
- **Standard Diluent (1x):** A buffered protein base with stabilizer (2 ml).
- **Wash Buffer Concentrate (20x):** A 20-fold concentrated buffered surfactant (30 ml, 2 bottles).
- **SP Conjugate (100x):** A 100-fold concentrate (80 µl).
- **Chromogen Substrate (1x):** A stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution (1x):** A 0.5 N hydrochloric acid solution to stop the chromogen substrate reaction (12 ml).

Storage Condition

- Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.
- Store Standard, SP Conjugate, and Biotinylated Antibody at -20°C.
- Store Microplate, Diluent Concentrate (10x), Standard Diluent (1x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C.
- 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.

Other Supplies Required

- 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

Sample Collection, Preparation, and Storage

- **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.

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

Refer to Dilution Guidelines for further instruction.

Guidelines for Dilutions of 100-fold or Greater (*for reference only; please follow the insert for specific dilution suggested)*	
100x	**10000x**
A) 4 µl sample: 396 µl buffer (100x) = 100-fold dilution *Assuming the needed volume is less than or equal to 400 µl.*	A) 4 µl sample : 396 µl buffer (100x) B) 4 µl of A : 396 µl buffer (100x) = 10000-fold dilution *Assuming the needed volume is less than or equal to 400 µl.*
1000x	**100000x**
A) 4 µl sample : 396 µl buffer (100x) B) 24 µl of A : 216 µl buffer (10x) = 1000-fold dilution *Assuming the needed volume is less than or equal to 240 µl.*	A) 4 µl sample : 396 µl buffer (100x) B) 4 µl of A : 396 µl buffer (100x) C) 24 µl of B : 216 µl buffer (10x) = 100000-fold dilution *Assuming the needed volume is less than or equal to 240 µl.*

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use.
- **MIX Diluent Concentrate (10x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.

Dilute the MIX Diluent Concentrate 10-fold with reagent grade water to produce a 1x solution. Store for up to 30 days at 2-8°C.

- **Human Annexin A7 Standard:** Reconstitute the Human Annexin A7 Standard (80 ng) with 1 ml of **Standard Diluent** to generate an 80 ng/ml standard stock solution. Allow the vial to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting from the standard stock solution (80 ng/ml) 2-fold with equal volume of **MIX Diluent** to produce 40, 20, 10, 5, 2.5, 1.25, and 0.625 ng/ml solutions. MIX Diluent serves as the zero standard (0 ng/ml). Aliquot remaining stock solution to limit repeated freeze-thaw cycles. This solution should be stored at -20°C and **used within 30 days**.

Standard Point	Dilution	[Annexin A7] (ng/ml)
P1	1 part Standard (80 ng/ml) + 1 part MIX Diluent	40
P2	1 part P1 + 1 part MIX Diluent	20
P3	1 part P2 + 1 part MIX Diluent	10
P4	1 part P3 + 1 part MIX Diluent	5
P5	1 part P4 + 1 part MIX Diluent	2.5
P6	1 part P5 + 1 part MIX Diluent	1.25
P7	1 part P6 + 1 part MIX Diluent	0.625
P8	MIX Diluent	0.0

- **Biotinylated Human Annexin A7 Antibody (50x):** Spin down the antibody briefly and dilute the desired amount of the antibody 50-fold with MIX Diluent to produce a 1x solution. The undiluted antibody should be stored at -20°C.
- **Wash Buffer Concentrate (20x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Wash Buffer Concentrate 20-fold with reagent grade water to produce a 1x solution.
- **SP Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 100-fold with MIX Diluent to produce a 1x solution. The undiluted conjugate should be stored at -20°C.

Assay Procedure

- Prepare all reagents, standard solutions, and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-25°C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch

securely to minimize exposure to water vapor and store in a vacuum desiccator.

- 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.
- 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.
- 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.
- Wash the microplate as described above.
- 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.
- Wash the microplate as described above.
- 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 20 minutes or until the optimal blue color density develops.
- Add 50 μl of Stop Solution to each well. The color will change from blue to yellow. Gently tap plate to ensure thorough mixing. Break any bubbles that may have formed.
- 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.

Data Analysis

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

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

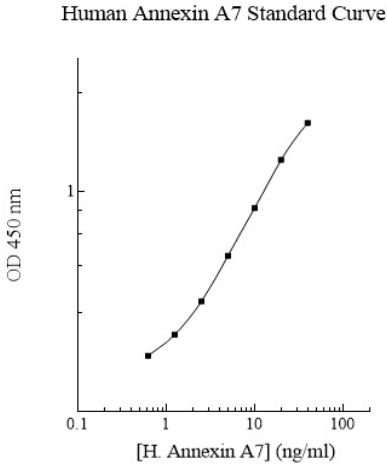
Typical Data

- The typical data is provided for reference only. Individual laboratory means may vary from the values listed. Variations between laboratories may be caused by technique differences.

Standard Point	ng/ml	OD	Average OD
P1	40	2.081 2.007	2.044
P2	20	1.413 1.361	1.387
P3	10	0.857 0.819	0.838
P4	5.0	0.522 0.496	0.509
P5	2.5	0.319 0.313	0.316
P6	1.25	0.228 0.218	0.223
P7	0.625	0.181 0.177	0.179
P8	0.0	0.131 0.129	0.130

Standard Curve

- The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.



Reference Value

- These cell lines were tested in house (n=10). The cell line averages are provided for reference only.

Cell Culture Lysate	Dilution Factor	Average Value (ng/mg cell lysate)
293T (human embryonic kidney)	20x	41.61
A549 (human adenocarcinoma)	20x	17.95
HeLa (human cervical cancer)	20x	9.041
Jurkat E6-1 (human T-cell leukemia)	40x	62.04

Performance Characteristics

- This assay recognizes both natural and recombinant human annexin A7.
- The minimum detectable dose of human annexin A7 as calculated by 2SD from the mean of a zero standard was established to be 0.45 ng/ml.
- Intra-assay precision was determined by testing three reference control samples twenty times in one assay.
- Inter-assay precision was determined by testing three reference control 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 (%)	4.8%	5.7%	5.0%	7.5%	8.6%	8.2%
Average CV (%)	5.2%			8.1%		

Recovery

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

Linearity

- Lysate samples were serially diluted to test for linearity.

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

Cross-Reactivity

Protein	Cross-Reactivity (%)
Annexin A5	<2%

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

Troubleshooting

Issue	Causes	Course of Action
Low Precision	Use of improper 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 empty 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. • Competitive ELISA: If samples generate OD values lower 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.

References

(1) Yuan H *et al.* (2019) *Am J Trans Res*. 11(5):2754-2764.
(2) Jin Y *et al.* (2013) *BMC Cancer*. 13(522).
(3) Leighton X *et al.* (2018) *PLoS ONE*. 13(10):e0205837.
(4) Ye W *et al*. (2018) *Onc Lett*. 15:9836-9844.

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