ab283992 – Human ANNEXIN-A13/ANXA13 ELISA Kit

For the quantitative measurement of ANNEXIN-A13/ANXA13 in human plasma, serum, cell culture 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/ab283992

Storage and Stability: Store kit at +4°C immediately upon receipt, apart from the SP Conjugate, Biotinylated Antibody and Standard, which should be stored at -20°C.

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
Chromogen Substrate	7 mL	4°C
Human ANNEXIN-A13/ANXA13 Microplate	1 vial	4°C
ANNEXIN-A13/ANXA13 Standard	96 wells	-20°C
Sealing Tapes	3	N/A
Stop Solution	11 mL	4°C
50X Biotinylated Human ANNEXIN-A13/ANXA13 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: If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Diluent M Concentrate 1:10 with reagent grade water to produce a 1x solution. Store for up to 30 days at 2-8°C.

50X Biotinylated Human ANNEXIN-A13/ANXA13 Antibody: Spin down the antibody briefly and dilute the desired amount of the antibody 1:50 with 1X Diluent M to produce a 1x solution. The undiluted antibody should be stored at -20°C.

20X Wash Buffer Concentrate: If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Wash Buffer Concentrate 1:20 with reagent grade water to produce a 1x solution.

100X Streptavidin-Peroxidase Conjugate (100x): Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with 1X 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 Human ANNEXIN-A13/ANXA13 Standard vial to prepare a 200 ng/ml Stock Standard.

- First consult the ANNEXIN-A13/ANXA13 Standard vial to determine the mass of protein in the vial.
- Calculate the appropriate volume of Standard Diluent to add when resuspending the ANNEXIN-A13/ANXA13 Standard vial to produce a 200 ng/ml ANNEXIN-A13/ANXA13 Stock Standard by using the following equation:
    - C_S = Starting mass of ANNEXIN-A13/ANXA13 Standard (see vial label) (µg)
    - C_F = The 200 ng/ml ANNEXIN-A13/ANXA13 Stock Standard final required concentration
    - V_D = Required volume of Standard Diluent for reconstitution (µL)
    - Calculate total required volume Standard Diluent 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**.

CS = 80 ng of ANNEXIN-A13/ANXA13 Standard in vial

CF = 200 ng/mL ANNEXIN-A13/ANXA13 Stock Standard final concentration

VD = Required volume of Standard Diluent for reconstitution (80 ng / 200 ng/mL) x 1,000 = 400 µL

- Reconstitute the ANNEXIN-A13/ANXA13 Standard vial by adding the appropriate calculated amount VD of **Standard Diluent** to the vial to generate the 200 ng/mL ANNEXIN-A13/ANXA13 Stock Standard. Mix gently and thoroughly.
- Allow the reconstituted 200 ng/mL ANNEXIN-A13/ANXA13 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 M** to tubes #1 – 8.
- To prepare Standard #1, add 120 µL of the Stock Standard 200 ng/mL 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 M serves as the zero standard (0 ng/mL).

Standard #	Volume to dilute (µL)	Volume 1X Diluent M (µL)	ANNEXIN-A13/ANXA13 (ng/ml)
1	120 µL stock standard (200 ng/mL)	120	100.00
2	120 µL Standard #1	120	50.00
3	120 µL Standard #2	120	25.0
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. 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. 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 Culture Supernatants: Centrifuge cell culture media at 1500 rpm for 10 minutes at 4°C to remove debris and collect supernatants. Samples can be stored at -80°C. 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. Re-suspend pellet in ice-cold Lysis Buffer (10 mM Tris, pH8.0, 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.

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. 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).
2. 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.
3. Add 50 µl of Human ANNEXIN-A13/ANXA13 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.
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.
5. Add 50 µl of Biotinylated Human ANNEXIN-A13/ANXA13 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 2 hours.
6. Wash the microplate as described above.
7. 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.
8. Wash the microplate as described above.
9. 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.
10. 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.
11. 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	100	1.837 1.785	1.811
2	50	1.363 1.321	1.342
3	25	0.838 0.803	0.821
4	12.5	0.539 0.522	0.531
5	6.25	0.377 0.350	0.364
6	3.125	0.250 0.247	0.249
7	1.563	0.186 0.183	0.185
8	0.0	0.125 0.123	0.124

PERFORMANCE CHARACTERISTICS

1. The minimum detectable dose of human ANNEXIN-A13/ANXA13 as calculated by 2SD from the mean of a zero standard was established to be 1.1 ng/ml.
2. Intra-assay precision was determined by testing three samples twenty times in one assay.
3. Inter-assay precision was determined by testing three samples in twenty assays.

	Intra-Assay Precision	Inter-Assay Precision
CV (%)	4.0	9.9

RECOVERY

Standard Added Value	6.25 – 50 ng/ml
Recovery %	89 – 111
Average Recovery %	97

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

Technical Support

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