Annexin A1 (human) ELISA Kit

Item No. 501550

www.caymanchem.com
Customer Service 800.364.9897
Technical Support 888.526.5351
1180 E. Ellsworth Rd · Ann Arbor, MI · USA

TABLE OF CONTENTS

GENERAL INFORMATION

Materials Supplied

Item Number	Item	96 wells Quantity/Size
401552	Anti-Annexin A1 (human) ELISA Strip Plate	1 plate
401551	Anti-Annexin A1 (human) HRP Conjugate	1 vial/1.5 ml
401554	Annexin A1 (human) ELISA Standard	1 vial/100 ng
400054	Immunoassay Buffer B Concentrate (10X)	1 vial/10 ml
400062	Wash Buffer Concentrate (400X)	1 vial/5 ml
400035	Polysorbate 20	1 vial/3 ml
400074	TMB Substrate Solution	1 vial/12 ml
10011355	HRP Stop Solution	1 vial/12 ml
400012	96-Well Cover Sheet	1 cover

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

The reagents in this kit have been tested and formulated to work exclusively with Cayman's Annexin A1 (human) ELISA Kit. This kit may not perform as described if any reagent or procedure is replaced or modified. The Stop Solution provided with this kit is an acid solution. Please wear appropriate personal protection equipment (*e.g.*, safety glasses, gloves, and lab coat) when using this material.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Email: techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed at 4°C and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A orbital microplate shaker.
2. A plate reader capable of measuring absorbance at 450 nm.
3. Adjustable pipettes and a repeating pipettor.
4. A source of pure, glass distilled, or HPLC-grade water. *NOTE: Ultra-Pure water is available for purchase from Cayman (Item No. 400000)*

INTRODUCTION

Background

Annexin A1 is a natural anti-inflammatory protein produced by neutrophils and monocytes. The glucocorticoid-induced production and release of Annexin A1 is the primary means by which glucocorticoids function as anti-inflammatory agents. Annexin A1 inhibits the synthesis of pro-inflammatory eicosanoids by suppressing the function of secretory phospholipase A_2 ($sPLA_2$). This, in turn, limits the recruitment of neutrophils into inflammatory sites and down-regulates the production of pro-inflammatory mediators by those neutrophils that enter inflammatory sites. Annexin A1 also functions in the resolution of inflammation by inducing neutrophil apoptosis, and promoting neutrophil clearance (efferocytosis) by macrophages. The pro-resolving functions of Annexin A1 are mediated via binding to FPR2/ALX, a receptor it shares with the specific pro-resolving mediators lipoxin A_4 (LXA_4) and resolving D1 (RvD1).

About This Assay

Cayman's Annexin A1 (human) ELISA Kit is a sandwich assay which can be used to measure human Annexin A1 in tissue culture media, serum and plasma. The standard curve spans the range of 0.20-20 ng/ml.

Principle of the Assay

This sandwich assay is based on a double-antibody 'sandwich' technique. Each well of the microwell plate supplied with the kit has been coated with a monoclonal antibody specific for Annexin A1 (mouse anti-human Annexin A1). This antibody will bind any Annexin A1 introduced into the well. Standards and samples are incubated on the antibody-coated plate, and the plate is then rinsed before addition of an HRP-labeled Annexin A1 polyclonal antibody to detect the captured Annexin A1. The two antibodies form a 'sandwich' by binding to different locations on the Annexin A1 protein. The concentration of the analyte is determined by measuring the enzymatic activity of HRP using the chromogenic substrate 3,3',5,5'-tetramethylbenzidine (TMB). After a sufficient period, the reaction is stopped with acid, forming a product with a distinct yellow color that can be measured at 450 nm. The intensity of this color, determined spectrophotometrically, is directly proportional to the amount of bound conjugate, which in turn is proportional to the concentration of the Annexin A1.

$$\text{Absorbance} \propto [\text{Anti-Annexin A1 HRP}] \propto [\text{Annexin A1}]$$

A schematic of this process is shown in Figure 1, below

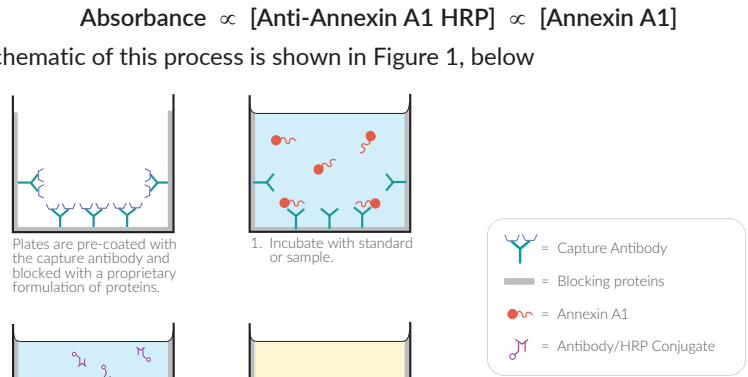


Figure 1. Schematic of the ELISA

Definition of Key Terms

Lower Limit of Quantification (LLOQ): is defined as the lowest standard concentration in which absorbance (450 nm) – (1.64 x S.D.) is higher than the blank value of absorbance (450 nm) + (1.64 x S.D.).

Lower Limit of Detection (LLOD): the smallest measure that can be detected with reasonable certainty for a given analytical procedure. The LLOD is defined as two standard deviations away from the mean zero value.

Dynamic Range: the range in which the analyte is reliably quantifiable.

Standard Curve: a plot of the absorbance values *versus* concentration of a series of wells containing various known amounts of free analyte.

PRE-ASSAY PREPARATION

Buffer Preparation

Store all diluted buffers at 4°C; they should be stable for about two months.

1. **Immunoassay Buffer B Preparation**

   Dilute the contents of one vial of Immunoassay Buffer B Concentrate (10X) (Item No. 400054) with 90 ml of deionized water. Be certain to rinse the vial to remove any salts that may have precipitated. *NOTE: It is normal for the concentrated buffer to contain crystalline salts after thawing. These will completely dissolve upon dilution.*

2. **Wash Buffer Preparation**

   5 ml vial Wash Buffer (96-well kit; Item No. 400062): Dilute to a total volume of 2 liters with deionized water and add 1 ml of Polysorbate 20 (Item No. 400035). *NOTE: Polysorbate 20 is a viscous liquid and cannot be measured by a regular pipette. A positive displacement pipette or a syringe should be used to deliver small quantities accurately.*

Sample Preparation

Sample Collection and Storage

In general, samples should be clarified by centrifugation to remove any particulates that could interfere in the assay.

NOTE: Published literature suggests that Annexin A1 tends to form calcium-dependent dimers that could potentially result in inaccurate readings in this ELISA. Cayman scientists have noted Annexin A1 dimerization in human serum but not in human plasma.

Sample Dilution

All human plasma, serum samples, and cell culture supernatants MUST be diluted at least 1:2 with Immunoassay Buffer B prior to use in this assay. A minimum volume of 200 µl of each diluted sample is needed to run the samples in duplicate in the assay; for convenience, we recommend that you prepare 250 µl of each diluted sample by combining 125 µl of sample with 125 µl of Immunoassay Buffer B (1X).

Sample Matrix Properties

Linearity

Human plasma, human serum, and supernatants from apoptotic human neutrophils were analyzed at multiple dilutions using the Annexin A1 (human) ELISA Kit. The results are shown in tables 1-3 below and on page 12.

Dilution	Concentration (ng/ml)	Dilutional Linearity (%)
2	10.3	100
4	11.2	108
8	10.8	104
16	10.5	101

Table 1: Dilutional linearity of human plasma in the Annexin A1 (human) ELISA Kit.

Dilution	Concentration (ng/ml)	Dilutional Linearity (%)
2	24.5	100
4	23.2	95
8	24.8	101
16	25	102

Table 2: Dilutional linearity of spiked human serum in the Annexin A1 (human) ELISA Kit.

Dilution	Concentration (ng/ml)	Dilutional Linearity (%)
2	14.6	100
4	15.1	103
8	13.8	94
16	14.3	98

Table 3: Dilutional linearity of supernatants of apoptotic neutrophils in RPMI/10% FBS (cell culture medium) in the Annexin A1 (human) ELISA Kit.

Spike and Recovery

Human plasma, human serum and cell culture supernatants were spiked with Annexin A1, diluted as described in the Sample Preparation section and analyzed using the Annexin A1 (human) ELISA Kit. The results are shown in table 4.

Sample	Spike Concentration (ng/ml)	% Recovery	Minimum Recommended Dilution
Plasma	20	95	1:2
	200	113	
	2,000	130	
Serum	20	105	1.2
	200	112	
	2,000	132	
Cell Culture Supernatants	20	121	1:2
	200	115	
	2,000	139	

Table 4: Spike and recovery of Annexin A1 from sample matrices.

Parallelism

To assess parallelism, human plasma, serum, and cell culture supernatant samples were analyzed at multiple dilutions by the Annexin A1 (human) ELISA Kit. Absorbance (450 nm) were plotted as a function of the sample dilution. The results are shown in **Figure 2**, below. Parallelism of the curves demonstrates that the protein binding characteristics are similar enough to allow the accurate determination of native protein levels.

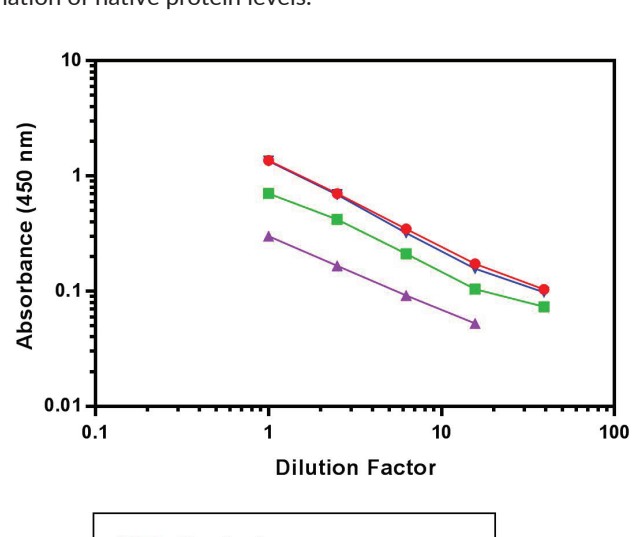


Figure 2. Parallelism of sample matrices in the Annexin A1 (human) ELISA Kit.

ASSAY PROTOCOL

Preparation of Assay-Specific Reagents

Annexin A1 (human) ELISA Standard (Item No. 401554)

Reconstitute the lyophilized Annexin A1 (human) ELISA Standard (Item No. 401554) with 0.5 ml of Immunoassay Buffer B. Mix gently. The concentration of this solution is 200 ng/ml. Prepare all subsequent dilutions of the Annexin A1 standard using polypropylene tubes, **do not use glass**.

To prepare the standards: Obtain seven clean test tubes and label them #1 through #7. Aliquot 900 µl Immunoassay Buffer B into tubes #1 and 600 µl Immunoassay Buffer B into tubes #2-7. Transfer 100 µl of the bulk standard (200 ng/ml) to tube #1 and mix thoroughly. Serially dilute the standard by removing 400 µl from the stock standard vial #1 and placing it in tube #2; mix thoroughly. Repeat this process for tubes #3-6. Do not add any Annexin A1 to tube #7. This tube is the background control.

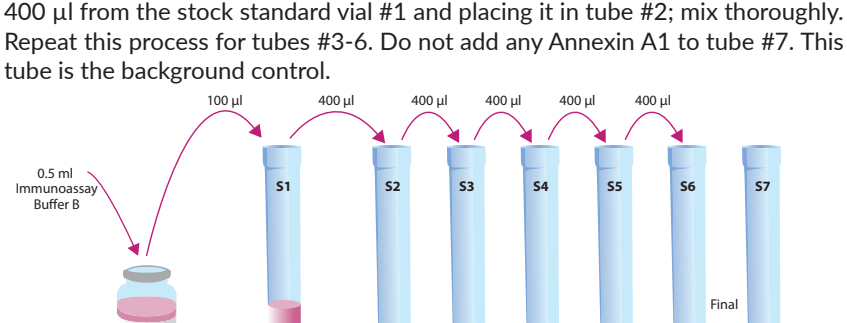


Figure 3: Preparation of the Annexin A1 (human) Standards.

Anti-Annexin A1 HRP Conjugate (Item No. 401551)

This reagent is supplied as a concentrated (10X) stock solution of Anti-Annexin A1 (human) antibody conjugated to horseradish peroxidase (HRP). On the day of the assay, prepare a working solution of the HRP conjugate (Item No. 401551). For a full plate, dilute 1.2 ml of HRP Conjugate into 10.8 ml of 1X Immunoassay Buffer B; for a half plate, dilute 0.6 ml of HRP Conjugate into 5.4 ml of 1X Immunoassay Buffer B.

Plate Set Up

The 96-well plate(s) included with this kit is supplied ready to use. It is not necessary to rinse the plate(s) prior to adding the reagents. *NOTE: If you do not need to use all the strips at once, place the unused strips back in the plate packet and store at 4°C.* **Be sure the packet is sealed with the desiccant inside.**

Each plate or set of strips must contain a seven point standard curve run in duplicate. NOTE: Each assay must contain this minimum configuration to ensure accurate and reproducible results. Each sample should be assayed at a minimum of two dilutions and each dilution should be assayed in duplicate. For statistical purposes, we recommend assaying samples in triplicate.

A suggested plate format is shown in Figure 4, below. The user may vary the location and type of wells present as necessary for each individual experiment. We suggest you record the contents of each well on the template sheet provided (see page 25).

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1	1	1	9	9	17	17	25	25	33	33
B	S2	S2	2	2	10	10	18	18	26	26	34	34
C	S3	S3	3	3	11	11	19	19	27	27	35	35
D	S4	S4	4	4	12	12	20	20	28	28	36	36
E	S5	S5	5	5	13	13	21	21	29	29	37	37
F	S6	S6	6	6	14	14	22	22	30	30	38	38
G	S7	S7	7	7	15	15	23	23	31	31	39	39
H	Blk	Blk	8	8	16	16	24	24	32	32	40	40

Blk - Blank
S1-S7 - Standards 1-7
1-40 - Samples

Figure 4. Sample plate format

Performing the Assay

Pipetting Hints

- Use different tips to pipette each reagent.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

NOTE: Perform all assay steps in the order given and without appreciable delays between steps. Pipetting samples should not extend beyond ten minutes to avoid assay drift. TMB Substrate Solution and Stop Solution should be added in the same sequence.

Addition of Standards, Samples, and First Incubation

1. Pipette 100 µl of the Annexin A1 ELISA Standards into the appropriate wells on the plate.
2. Pipette 100 µl of diluted samples into each well. *NOTE: Human plasma and serum samples and cell culture supernatants must be diluted at least 1:2 with Immunoassay Buffer B (see page 13).*
3. Cover the plate with the enclosed 96-well plate cover sheet and incubate for two hours at room temperature on an orbital shaker.

Addition of Anti-Annexin A1 HRP Conjugate and Second Incubation

1. Empty the wells and rinse five times with Wash Buffer. Each well should be filled completely with Wash Buffer during each wash. After the last wash, invert the plate and gently tap on absorbent paper to remove any residual Wash Buffer.
2. Prepare a 1X working solution of the Anti-Annexin A1 (human) HRP-Conjugate (Item No. 401551) as directed previously (see page 16).
3. Add 100 µl of the anti-Annexin A1 (human) HRP-Conjugate working solution to each well of the plate.
4. Cover the plate with the enclosed 96-well plate cover sheet and incubate for one hour at room temperature on an orbital shaker.

Development of the Plate

1. Empty the wells and rinse five times with Wash Buffer. Each well should be filled completely with Wash Buffer during each wash. After the last wash, invert the plate and gently tap on absorbent paper to remove any residual Wash Buffer.
2. Add 100 µl of TMB Substrate Solution (Item No. 400074) to each well of the plate.
3. Cover the plate with the enclosed 96-well plate cover sheet and incubate for 30 minutes at room temperature on an orbital shaker.
4. DO NOT WASH THE PLATE. Add 100 µl of HRP Stop Solution (Item No. 10011355) to each well of the plate. Blue wells should turn yellow and colorless wells should remain colorless. *NOTE: The stop solution in this kit contains an acid. Wear appropriate protection and use caution when handling this solution.*

Reading the Plate

1. Wipe the bottom of the plate with a clean tissue to remove fingerprints, dirt, etc.
2. Read the plate at a wavelength of 450 nm.

ANALYSIS

Many plate readers come with data reduction software that plots data automatically. If your plate reader can analyze the data, we recommend using a quadratic curve fit. Alternatively, a spreadsheet program can be used.

Calculations

Plotting the Standard Curve and Determining the Sample Concentration

Using computer reduction software, plot absorbance (linear y-axis) versus concentration (linear x-axis) for standards (S1-S7) and fit the data with a quadratic, or alternatively a four-parameter logistic curve equation. Using the equation of the curve, calculate the concentration of Annexin A1 in each sample making sure to correct for any sample dilution.

Performance Characteristics

Sample Data

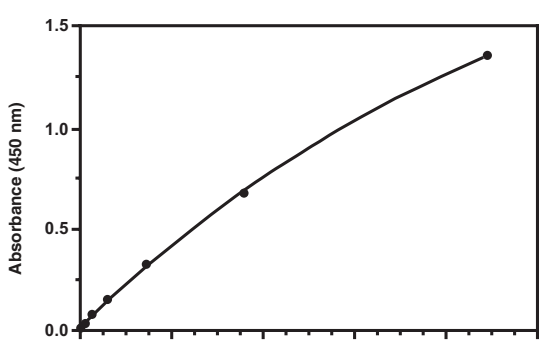
The standard curve presented here is an example of the data typically produced with this kit; however, your results will not be identical to these. You **must** run a new standard curve. Do not use the data below to determine the values of your samples. Your results could differ substantially.

Annexin A1 Standards (ng/ml)	Blank Subtracted Absorbance 450 nm	%CV Intra-Assay Precision	%CV Inter-Assay Precision
20	1.352	3	0.1
8	0.675	1.5	1.5
3.2	0.322	4.4	4.7
1.28	0.147	2.2	9.4
0.51	0.069	11.4	14.5
0.2	0.03	17.3	46.7*
0	0.007**		

**Data at this region of the standard curve should be used with caution.*

***Blank is comprised of only TMB, Blank Subtracted zero point value is indicative of any minimal non-specific binding. Zero point should be plotted on standard curve.*

Table 5. Typical results



Assay Range = 0.20-20 ng/ml
Dynamic Range = 0.20-20 ng/ml
Sensitivity (defined as LLOQ) = 0.2 ng/ml
Lower Limit of Detection = 0.18 ng/ml

The sensitivity was derived from the standard curve shown above.
The standard was diluted with ELISA Buffer.

Figure 6. Typical standard curve

Precision:

Intra-assay precision was determined by analyzing 24 replicates of three matrix controls (human plasma samples) in a single assay.

Matrix Control (ng/ml)	%CV
10.3	9.7
6.8	5.9
4.5	16.2

Table 6. Intra-assay precision

Inter-assay precision was determined by analyzing replicates of three matrix controls (human plasma samples) in separate assays, spanning across several days.

Matrix Control (ng/ml)	%CV
10.8	8.7
7.1	5.9
4.1	5.7

Table 7. Inter-assay precision

RESOURCES

Troubleshooting

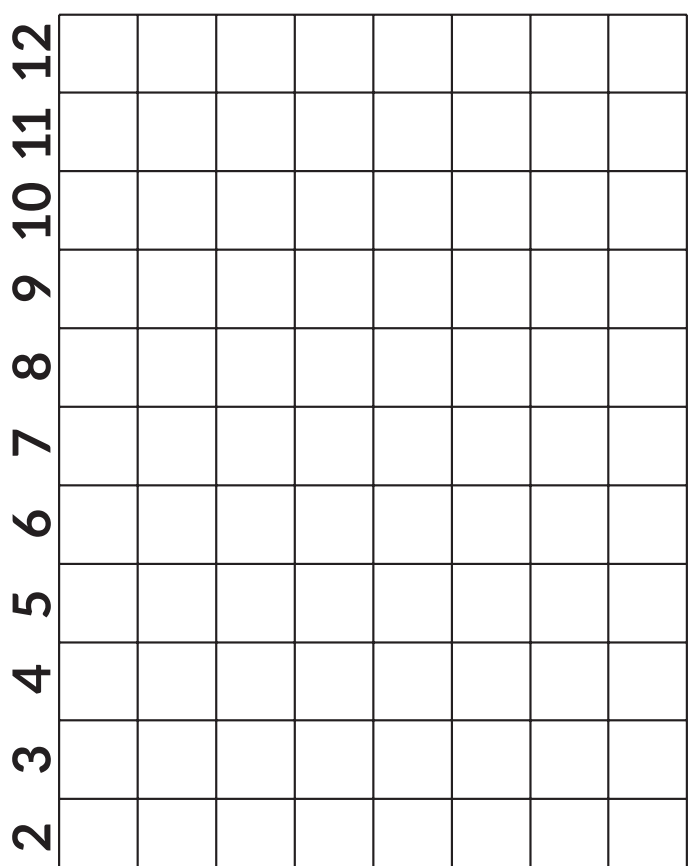
Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates	A. Trace organic contaminants in the water B. Poor pipetting/ technique	A. Replace activated carbon filter or change source of UltraPure water
Poor development (low signal) of standard curve	HRP conjugate was diluted into ELISA buffer too long before use	Make up fresh HRP conjugate and re-run the assay

Procedure	TMB Blank	Standards/Samples
Apply Annexin A1 (human) Standards/ Controls to appropriate wells	--	100 µl
Cover plate and incubate for 2 hours, shaking at room temperature	→	→
After 2 hours of standard and sample incubation, aspirate and wash plate 5 x full well volume (~300 µl) with 1X Wash Buffer	→	→
Apply HRP-Conjugate	--	100 µl
Cover plate and incubate for 1 hour shaking at room temperature	→	→
After 1 hour of HRP-Conjugate incubation, aspirate and wash plate 5 x full well volume (~300 µl) with 1X Wash Buffer	→	→
Apply TMB Substrate Solution	100 µl	100 µl
Cover plate and incubate for 30 minutes shaking at room temperature	→	→
After 30 minutes of substrate incubation, DO NOT WASH PLATE. Apply HRP Stop Solution	100 µl	100 µl
Read plate using at a wavelength of 450 nm	→	→

Table 8. Annexin A1 Assay Summary

References

1. Yang, Y.H., Morand,E., and Leech, M. Annexin A1: Potential for glucocorticoid sparing in RA. *Nat. Rev. Rheumatol.* **9(10)**, 595-603 (2013).
2. Gungor, H.E., Tahan, F., Gokahmetoglu, S., *et al.* Decreased levels of lipoxin A4 and annexin A1 in wheezy infants. *Int. Arch. Allergy. Immunol.* **163(3)**, 193-197 (2014).
3. Sugimoto, M.A., Vago, J.P., Teixiera, M.M., *et al.* Annexin A1 and the resolution of inflammation: Modulation of neutrophil recruitment, apoptosis, and clearance. *J. Immunol. Res.* **2016**, 8239258 (2016).
4. Bozinovski, S., Anthony, D., and Vlahos, R. Targeting pro-resolution pathways to combat chronic inflammation in COPD. *J. Thorac. Dis.* **6(11)**, 1548-1556 (2014).
5. Hirata, A., Corcoran, G.B., and Hirata, F. Carcinogenic heavy metals replace Ca^{2+} for DNA binding and annealing activities of mono-ubiquitinated annexin A1 homodimer. *Toxicol. Appl. Pharmacol.* **248(1)**, 45-51 (2010).



NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.