



[Go to Product page](#)

Datasheet for ABIN454434  
**ATOH1 ELISA Kit**

### Overview

Quantity:	96 tests
Target:	ATOH1
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	0.78-50 ng/mL
Minimum Detection Limit:	0.78 ng/mL
Application:	ELISA

### Product Details

Purpose:	This immunoassay kit allows for the use in vitro quantitative determination of human Apolipoprotein H, Apo-H concentrations in cell culture supernates, serum, plasma and other biological fluids.
Sample Type:	Cell Culture Supernatant, Plasma, Serum
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This assay recognizes recombinant and natural human Apo-H.
Cross-Reactivity (Details):	No significant cross-reactivity or interference was observed.
Sensitivity:	< 1.95 ng/mL The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest detectable concentration that could be differentiated from zero.

## Product Details

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Characteristics:	Homo sapiens,Human,Protein atonal homolog 1,Class A basic helix-loop-helix protein 14,bHLHa14,Helix-loop-helix protein hATH-1,hATH1,ATOH1,ATH1,BHLHA14
Components:	Reagent (Quantity): Assay plate (1), Standard (2), Sample Diluent (1x20ml), Assay Diluent A (1x10ml), Assay DiluentB 1 x 10ml Detection Reagent A (1x120µl), Detection Reagent B (1x120µl), Wash Buffer(25 x concentrate) (1x30ml), Substrate (1x10ml), Stop Solution (1x10ml), Plate sealer for 96 wells (1x5)

## Target Details

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Target:	ATOH1
Alternative Name:	ATOH1 ( <a href="#">ATOH1 Products</a> )
Background:	<p>Apolipoprotein H (Apo-H), previously known as (beta2-glycoprotein I, beta-2 glycoprotein I), is a multifunctional apolipoprotein. One of its function is to bind cardiolipin. When bound the structure of cardiolipin and Apo-H both undergo large changes in structure. Within the structure of Apo-H is a stretch of positively charged amino acids, (protein sequence positions 282-287) Lys-Asn-Lys-Glu-Lys-Lys, are involved in phospholipid binding (See image on right). Apo-H has a complex involvement in agglutination, it appears to alter ADP mediated agglutination of platelets. Normally Apo-H assumes an anti-coagulation activity in serum (by inhibiting coagulation factors), however changes in blood factors can result of a reversal of that activity. Apo-H appears to completely inhibit serotonin release by the platelets and prevents subsequent waves of the ADP-induced aggregation. The activity of Apo-H appears to involve the binding of agglutinating, negatively charged compounds, and inhibits agglutination by the contact activation of the intrinsic blood coagulation pathway. Apo-H causes a reduction of the prothrombinase binding sites on platelets and reduces the activation caused by collagen when thrombin is present at physiological serum concentrations of Apo-H suggesting a regulatory role of Apo-H in coagulation. Apo-H also inhibits the generation of factor Xa in the presence of platelets. Apo-H also inhibits that activation of factor XIIa. In addition, Apo-H inhibits the activation of protein C blocking its activity on phosphatidylserine:phosphatidylcholine vesicles however once protein C is activated, Apo-H fails to inhibit activity. Since protein C is involved in factor Va degradation Apo-H indirectly inhibits the degradation of factor Va. This inhibitory activity was diminished by adding phospholipids suggesting the Apo-H inhibition of protein C is phospholipid competitive. This indicates that under certain conditions Apo-H takes on a procoagulation properties.</p>

## Application Details

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Sample Volume:	100 µL
Plate:	Pre-coated
Protocol:	<p>The microtiter plate provided in this kit has been pre-coated with an antibody specific to Apo-H. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for Apo-H and Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Then a TMB (3,3',5, 5' tetramethyl-benzidine) substrate solution is added to each well. Only those wells that contain Apo-H, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of Apo-H in the samples is then determined by comparing the O.D. of the samples to the standard curve.</p>
Reagent Preparation:	<p>Bring all reagents to room temperature before use. Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 30 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 750 mL of Wash Buffer. Standard - Reconstitute the Standard with 1.0 mL of Sample Diluent. This reconstitution produces a stock solution of 500 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making serial dilutions. The undiluted standard serves as the high standard (500 ng/mL). The Sample Diluent serves as the zero standard (0 ng/mL). Detection Reagent A and B - Dilute to the working concentration specified on the vial label using Assay Diluent A and B (1:100), respectively.</p>
Sample Collection:	<p>Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at approximately 1000 x g. Remove serum and assay immediately or aliquot and store samples at -20 °C or -80 °C. Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000 x g at 2 - 8 °C within 30 minutes of collection. Store samples at -20 °C or -80 °C. Avoid repeated freeze-thaw cycles. Cell culture supernates and other biological fluids - Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20 °C or -80 °C. Avoid repeated freeze-thaw cycles. Sample preparation - Serum/plasma samples require a 1000 fold dilution. Note: Serum, plasma, and cell culture supernatant samples to be used within 7 days may be stored at 2-8°C, otherwise samples must stored at -20 °C (≤ 3 months) or -80 °C (≤ 6 months) to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles. When performing the assay slowly bring samples to room temperature. It is recommended that all samples be assayed in duplicate. 3</p>

### Assay Procedure:

Allow all reagents to reach room temperature. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. Arrange and label required number of strips. Prepare all reagents, working standards and samples as directed in the previous sections.

1. Add 100  $\mu$ L of Standard, Blank, or Sample per well. Cover with the Plate sealer. Incubate for 2 hours at 37 °C.
2. Remove the liquid of each well, don't wash.
3. Add 100  $\mu$ L of Detection Reagent A working solution to each well. Cover with the Plate sealer. Incubate for 1 hour at 37°C. Detection Reagent A working solution may appear cloudy. Warm to room temperature and mix gently until solution appears uniform.
4. Aspirate each well and wash, repeating the process three times for a total of three washes. Wash by filling each well with Wash Buffer (350  $\mu$ L) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
5. Add 100  $\mu$ L of Detection Reagent B working solution to each well. Cover with a new Plate sealer. Incubate for 1 hours at 37 °C. 4
6. Repeat the aspiration/wash as in step
4. 7. Add 90  $\mu$ L of Substrate Solution to each well. Cover with a new Plate sealer. Incubate within 30 minutes at 37°C. Protect from light.
8. Add 50  $\mu$ L of Stop Solution to each well. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well at once, using a microplate reader set to 450 nm.

### Important Note:

1. Please carefully reconstitute Standards or working Detection Reagent A and B according to the instruction, and avoid foaming and mix gently until the crystals have completely dissolved. The reconstituted Standards can be used only once.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
3. It is recommended that no more than 32 wells be used for each assay run if manual pipetting is used since pipetting of all standards, specimens and controls should be completed within 5 minutes. A full plate of 96 wells may be used if automated pipetting is available.
4. Duplication of all standards and specimens, although not required, is recommended.
5. When mixing or reconstituting protein solutions, always avoid foaming.
6. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.

## Application Details

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7. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
8. Do not substitute reagents from one kit lot to another. Use only the reagents supplied by manufacturer.

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**Calculation of Results:** Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against 5 the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the Apo-H concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

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**Restrictions:** For Research Use only

## Handling

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**Handling Advice:**

1. The kit should not be used beyond the expiration date on the kit label.
2. Do not mix or substitute reagents with those from other lots or sources.
3. If samples generate values higher than the highest standard, further dilute the samples with the Assay Diluent and repeat the assay. Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
4. This assay is designed to eliminate interference by soluble receptors, ligands, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.

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**Storage:** 4 °C/-20 °C

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**Storage Comment:** The Standard, Detection Reagent A, Detection Reagent B and the 96-well strip plate should be stored at -20 °C upon being received. The other reagents can be stored at 4 °C.