

# HUMAN ASPROSIN ELISA KIT

FOR THE QUANTITATIVE DETERMINATION  
OF HUMAN ASPROSIN CONCENTRATIONS  
IN SERUM AND PLASMA



ALWAYS REFER TO LOT SPECIFIC PROTOCOL  
PROVIDED WITH EACH KIT FOR  
INSTRUCTIONS. PROTOCOL MUST BE  
READ BEFORE USING THIS PRODUCT.

FOR RESEARCH USE ONLY. NOT FOR USE IN  
DIAGNOSTIC PROCEDURES.

## PRODUCT INFORMATION:

**THIS KIT IS FOR ONE TIME USE ONLY.**

ELISA NAME	HUMAN ASPROSIN ELISA
Catalog No.	SK00229-06
Lot No.	
Formulation	96 T
Standard Range	1 - 32 nM/L
Sensitivity	0.1 nM/L
Sample Volume	100 µL per well
Sample Type	Serum, EDTA Plasma
Specificity	Human Asprosin
Calibration	Human Asprosin Recombinant
Dilution Factor	<b>Optimal dilutions should be determined by each laboratory for each application</b>
Intra-assay Precision	6 - 8%
Inter-assay Precision	8 - 12%
Storage	2 – 8° C
This kit contains sufficient materials to run 35 samples duplicated provided that assay is run according to protocol.	

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**DESCRIPTION**

This Human Asprosin /Fibrillin-1 (2732-2871) ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human Asprosin from serum and plasma in a sandwich ELISA format.

This immunoassay contains recombinant human Asprosin and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural Asprosin samples.

**ASSAY OVERVIEW**

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with a monoclonal antibody specific for human Asprosin. The capture antibody can bind to the human Asprosin in the standard and samples. After washing the plate of any unbound substances, a polyclonal antibody against human Asprosin is added to the wells. After another washing of the plate, Anti Goat IgG-HRP Conjugate is added. After the last wash to remove any unbound enzyme, a substrate solution is added to the wells and color develops in direct proportion to the amount of human Asprosin bound in the standard dilutions or samples. A standard curve can be established and sample values can be read off the standard curve.

**PROCEDURAL LIMITATIONS**

\_FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

\_This ELISA kit should not be used beyond the expiration date on the kit label.

\_Do not mix reagents with those from other lots or sources.

\_It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.

\_Each laboratory must determine the optimal dilution factors for the samples being assayed.

\_Any modifications in buffers, pipetting technique, washing technique, incubation time or temperature, as well as kit age can cause a change in signal.

\_Not all interfering factors have been tested in the immunoassay, therefore the possibility of interference cannot be excluded.

**COMPONENTS PROVIDED**

DESCRIPTION	CODE	QUANTITY
<b>Asprosin Microplate</b> - 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against human Asprosin.	<b>229-06-01</b>	<b>1 plate</b>
<b>Asprosin Standard</b> – refer to lot specific of human Asprosin in a buffered protein base with preservative; lyophilized.	<b>229-06-02</b>	<b>1 vial</b>
<b>Detection Antibody Concentrate</b> – 105 µL/vial of 100-fold concentrate of antibody solution against human Asprosin with preservative.	<b>229-06-03</b>	<b>1 vial</b>
<b>Positive Control</b> - one vial of human Asprosin; lyophilized.	<b>229-06-04</b>	<b>1 vial</b>
<b>Anti Goat IgG-HRP Conjugate</b> - 120 µL of 100-fold concentrated Anti IgG-HRP Conjugate.	<b>AGIGHRP</b>	<b>1 vial</b>
<b>Dilution Buffer</b> - 40 mL of buffered protein based solution with preservative.	<b>DB10</b>	<b>1 bottle</b>
<b>HRP Diluent Solution</b> - 12 mL of buffered protein based solution with preservative.	<b>DB8808C</b>	<b>1 bottle</b>
<b>Wash Buffer</b> - 50 mL of 10-fold concentrated buffered surfactant, with preservative.	<b>WB01</b>	<b>1 bottle</b>
<b>TMB Substrate Solution</b> - 11 mL of TMB substrate solution.	<b>TMB01</b>	<b>1 bottle</b>
<b>Stop Solution</b> - 11 mL of 0.5M HCl.	<b>S-STOP</b>	<b>1 bottle</b>
<b>Plate Sealer</b>	<b>EAPS</b>	<b>1</b>
<b>Plastic Pouch</b>	<b>P01</b>	<b>1</b>

**STORAGE**

**Unopened Kit:** Store at 2 – 8° C for up to 8 months. After receipt of ELISA kit, open box and remove Detection Antibody Concentrate and store at -20°C ~ -70°C until ready to use. For long-term storage, place unopened Standard and Positive Control in a freezer at -20° C or -70° C. Do not use kit past expiration date.

**ADDITIONAL MATERIALS REQUIRED**

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (250 – 300 rpm).
- Microplate washer or manifold dispenser.
- 100 mL and 500 mL graduated cylinders.
- Multi-channel Pipette, Pipettes and pipette tips.
- Deionized or distilled water.

**PRECAUTION**

This kit should be handled by those persons who have been trained in and can follow the principles of good laboratory practice. Wear protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken while handling solutions in this kit to avoid contact with skin or eyes, especially with the stop solution because it contains diluted hydrochloric acid. Wash immediately with water in case of contact on skin or eyes.

**SAMPLE COLLECTION AND STORAGE**

**Serum** - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at  $\leq -20^{\circ}$  C. Avoid repeated freeze-thaw cycles.

**Plasma** - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at  $\leq -20^{\circ}$  C. Avoid repeated freeze-thaw cycles.

**SAMPLE PREPARATION**

**Optimal dilutions should be determined by each laboratory for each application.**

Use polypropylene test tubes.

**REAGENT PREPARATION**

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 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of 1x Wash Buffer.

**Asprosin Standard** - Reconstitute the Asprosin standard with lot specific of Dilution Buffer. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 437.5  $\mu$ L of Dilution Buffer into tubes #1. Pipette 250  $\mu$ L of Dilution Buffer into tubes #2 to #6. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **32 nM/L** standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 nM/L). (The optional wide standard range may serve from 64nM/L to 0.5nM/L)

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
Stock	Powder	Lot specific	
# 1	Lot specific	Lot specific	32 nM/L
# 2	250 $\mu$ L of 2	250 $\mu$ L	16 nM/L
# 3	250 $\mu$ L of 3	250 $\mu$ L	8 nM/L
# 4	250 $\mu$ L of 4	250 $\mu$ L	4 nM/L
# 5	250 $\mu$ L of 5	250 $\mu$ L	2 nM/L
# 6	250 $\mu$ L of 6	250 $\mu$ L	1 nM/L

**Positive Control** – Reconstitute the Positive Control with 2.0 mL of **Dilution Buffer (DB10)**.

**Detection Antibody Concentrate** – Pipette 10.395 mL of **Dilution Buffer (DB10)** into a 15 mL centrifuge tube and transfer 105  $\mu$ L of 100-fold concentrated Detection Antibody stock solution to prepare 1x working solution.

**Anti Goat IgG-HRP Conjugate** – Pipette 11.88 mL of **HRP Diluent Solution (DB8808C)** into a 15 mL centrifuge tube and transfer 120  $\mu$ L of 100-fold concentrated stock solution to prepare working solution (**protect from light**).

**ELISA PROTOCOL**

Bring all reagents and samples to room temperature before the start of the assay. **Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.**

1. Prepare all reagents and working standards as directed in the previous sections.
2. Add 100  $\mu$ L per well of Dilution Buffer to Blank wells.

3. Add 100 µL of Standard dilutions, samples, or positive control per well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
4. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with 1x Wash Buffer (300 µL) using a squirt bottle, 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 µL of Detection Antibody working solution to each well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
6. Repeat the aspiration/wash as in step 4.
7. Add 100 µL of Anti Goat IgG-HRP working solution to each well. Cover with plate sealer. Incubate for 60 minutes on microplate shaker at room temperature. **Protect from light.**
8. Repeat the aspiration/wash as in step 4.
9. Add 100 µL of TMB Substrate Solution to each well. Incubate for refer to lot specific on microplate shaker at room temperature. **Protect from light.**
10. Add 100 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green, or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
11. Determine the optical density of each well using a microplate reader set to 450 nm.

**CALCULATION OF RESULTS**

Create a standard curve by plotting the log of the known concentrations of the standard dilutions (x-axis) versus the log of its corresponding O.D. (y-axis) and draw the best fit line through the points. It is recommended to use computer software capable of generating a log-log curve fit to more accurately quantify the standard dilutions. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

**TYPICAL STANDARD CURVE**

This standard curve is provided for demonstration only. A new standard curve should be generated for each set of samples assayed.

STANDARD (nM/L)	CORRECTED (450nm)
Blank	0 (0.104)
0.5 (optional)	0.028
1	0.055
2	0.146
4	0.271
8	0.630
16	1.222
32	3.450

**SPECIFICITY**

PROTEINS	CROSS-REACTIVITY (%)
Human Asprosin	100
Human Elastin	0
Human Irisin	0
Human Betatrophin	0

**SUMMARY OF ASSAY PROCEDURE**



