



**EAGLE**  
BIOSCIENCES

# **Human Asprosin ELISA Assay Kit**

Catalog Number:

**ASP31-K01 (1 x 96 wells)**

*For Research Use Only. Not for use in diagnostic procedures.*

v. 1.0 (16 AUG 24)

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## INTENDED USE

The Eagle Biosciences Human Asprosin ELISA Assay Kit is intended for the quantitative measurement of Asprosin in human serum/plasma, urine, and more. The Asprosin ELISA Assay Kit is for research use only and not to be used in diagnostic procedures.

## REAGENTS PROVIDED

Content	Volume
CP (Coated Plate)	96 well
S (Standard)	2 vial
DA-H 100x (Detect Antibody-HRP)	1 vial
SD (Standard Diluent)	6 mL/bottle
DD (Detect Antibody Diluent)	6 mL/bottle
AB (Assay Buffer 1x)	12 mL/bottle
TS (TMB Substrate)	12 mL/bottle
SS (Stop Solution)	12 mL/bottle
WB (Wash Buffer 10x)	50 mL/bottle
SF (Sealer Film)	6 pieces

Note: After the kit is opened, the stabilization period of each content is 30 days, so please use the kit within 30 days after opening.

## REAGENT PREPARATION

### Washing Buffer (1x) Preparation:

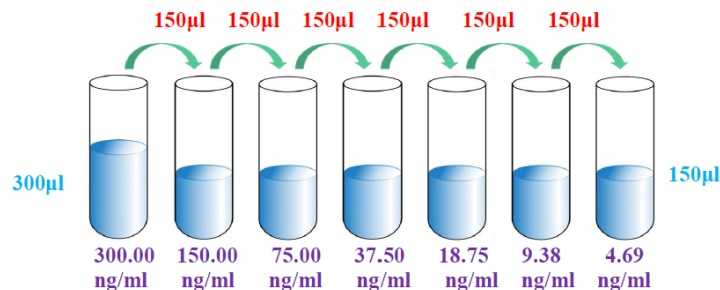
Pour entire contents (50 mL) of the **Washing Buffer Concentrate** (10x) into a clean 500 mL graduated cylinder. Bring to final volume of 500 mL with glass-distilled or deionized water. Transfer to a clean wash bottle and store at 2 to 25°C.

### Detect Antibody-HRP 1x Preparation:

Make a 1:100 dilution of the concentrated Detect Antibody solution with **DD (Detect Antibody Diluent)** in a clean plastic tube as needed according to the Standards and Samples.

### Standard Curve Preparation:

Reconstitute the Human Asprosin Standard with 100  $\mu$ L of deionized water. Add 30  $\mu$ L + 270  $\mu$ L SD (Standard Diluent) serves as the high standard (330 ng/mL). Pipette 150  $\mu$ L of SD into each tube. Use the high standard to produce 1:1 dilution series. Mix each tube thoroughly before the next transfer. SD serves as the zero standard (0 ng/mL).





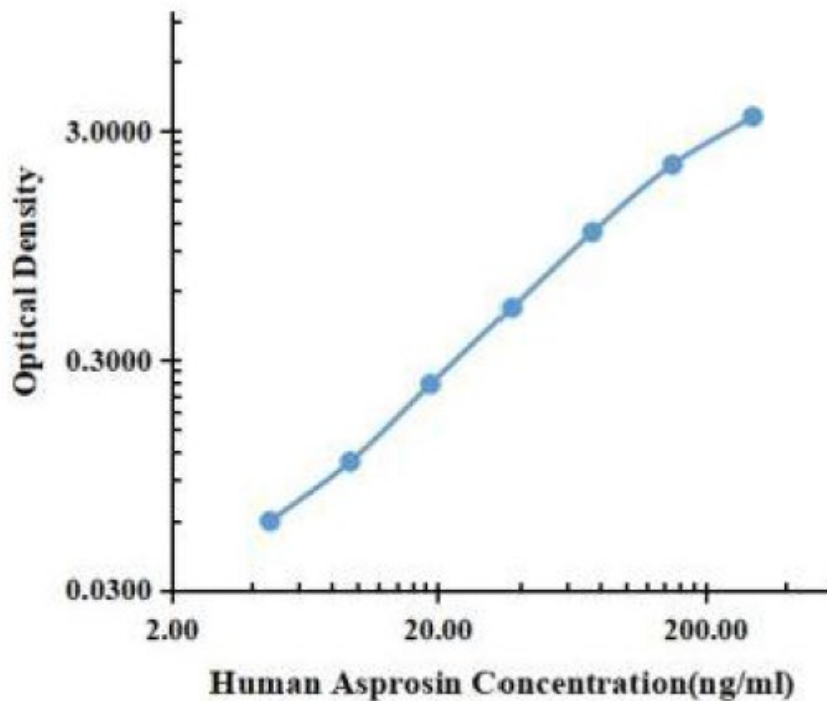
## ASSAY PROCEDURE

**Bring all reagents and samples to room temperature before use.**

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess **CP (Coated Plate)** strips from the plate frame, return them to the foil pouch and reseal.
3. Add 50  $\mu\text{L}$  of **AB (Assay Buffer)** to each well.
4. Add 50  $\mu\text{L}$  of **Standard or Sample** per well. Ensure reagent addition is uninterrupted and completed within 15 minutes.
5. Add 50  $\mu\text{L}$  of **DA-H (Detect Antibody-HRP)** to each well.
6. Cover with an **SF (Sealer Film)**. Incubate at room temperature (18 to 25°C) for 1 hour on a microplate **shaker** set to 500 rpm.
7. Aspirate each well and **wash**, repeating the process four times. Wash by filling each well with **WB (Washing Buffer 300  $\mu\text{L}$ )**. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining **WB (Washing Buffer)** by aspirating or decanting. Invert the plate and **blot** it against clean paper towels.
8. Add 100  $\mu\text{L}$  of **TS (TMB Substrate)** to each well. Incubate for 5-30 minutes at room temperature.
9. Add 100  $\mu\text{L}$  of **SS (Stop Solution)** to each well.
10. Determine the optical density within 30 minutes, using a microplate **reader** set to 450 nm corrected with 570 nm or 630 nm.

## TYPICAL DATA

**Human Asprosin Typical Standard**





ng/mL	O.D.		Average	Corrected
0.00	0.0407	0.0468	0.0438	
4.69	0.1037	0.1032	0.1035	0.0597
9.38	0.1406	0.1638	0.1522	0.1085
18.75	0.2698	0.2895	0.2797	0.2359
37.50	0.5256	0.5766	0.5511	0.5074
75.00	1.0950	1.1600	1.1275	1.0838
150.00	2.3230	2.0280	2.1755	2.1318
300.00	3.6020	3.3780	3.4900	3.4463

## SENSITIVITY

The minimum detectable dose (MDD) of human Asprosin is typically less than 0.78 ng/mL.

The MDD was determined by adding two standard deviations to the mean optical density value of ten zero standard replicates and calculating the corresponding concentration.

## PRECISION

### Intra-assay Precision (Precision within an assay)

Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

### Inter-assay Precision (Precision between assays)

Sample Number	Intra-assay Precision			Inter-assay Precision		
	S1	S2	S3	S1	S2	S3
	22	22	22	6	6	6
Average (ng/mL)	6.4	29.6	112.8	5.9	31.2	118.1
Standard Deviation	0.3	1.2	5.9	0.3	1.3	5.4
Coefficient of variation (%)	4.0	4.0	5.2	5.1	4.2	4.6

## SAMPLE VALUES

Serum/Plasma - Thirty samples from apparently healthy volunteers were evaluated for the presence of Asprosin in this assay. No medical histories were available for the donors.

Sample Matrix	Sample Evaluated	Range (pg/mL)	Detectable %	Mean of Detectable (pg/mL)
Serum	30	3.35 - 128.74	100%	34.32

## SAMPLE VALUES

n.d. = non-detectable. Samples measured below the sensitivity are considered to be non-detectable.

## SAMPLE DILUENT

If the concentration of the sample is too high, the sample can be diluted with **Sample Diluent**.



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