Human Asprosin ELISA Instructions

Cat: EH0032

Content

	CAT	Volume
❶ CP (Coated Plate)	EH0032CP	96 well
❷ S (Standard)	EH0032S	2 vial
❸ DA-H 100× (Detect Antibody-HRP)	EH0032DA-H	1 vial
❹ SD (Standard Diluent)	ESD04	6 ml/bottle
❺ DD (Detect Antibody Diluent)	EDD03	6 ml/bottle
❻ AB (Assay Buffer 1×)	EAB02	12 ml/bottle
❼ TS (TMB Substrate)	ETS01	12 ml/bottle
❽ SS (Stop Solution)	ESS01	12 ml/bottle
❾ WB (Wash Buffer 10×)	EWB01	50 ml/bottle
❿ SF (Sealer Film)	ESF01	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 (1×) Preparation

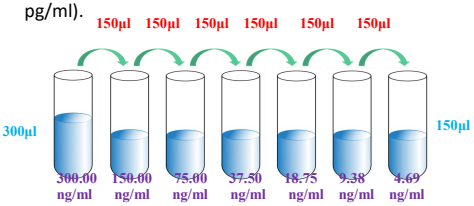
Pour entire contents (50 ml) of the **Washing Buffer Concentrate** (10×) into a clean 500 ml graduated cylinder. Bring to final volume of 500 ml with glass-distilled or deionized water. Transfer to a clean wash bottle and store at 2 to 25℃.

Detect Antibody-HRP 1× 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 (EH0032S) with 100 µl of deionized water. Add 30 µl EH0032S + 270 µl SD (Standard Diluent) serves as the high standard (300 ng/ml). Pipette 150 µl of SD into each tube. Use the high standard to produce a 1:1 dilution series. Mix each tube thoroughly before the next transfer. SD serves as the zero standard (0 pg/ml).

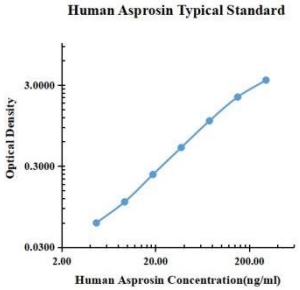


ASSAY PROCEDURE

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 µl of **AB** (Assay Buffer) to each well.
4. Add 50 µl of **Standard or sample** per well. Ensure reagent addition is uninterrupted and completed within 15 minutes.
5. Add 50 µl of **DA-H 1×** (Detect Antibody-HRP) to each well.
6. Cover with an **SF** (Sealer Film). Incubate at room temperature (18 to 25℃) for 1 hours on a microplate **shaker** set at 500 rpm.
7. Aspirate each well and **wash**, repeating the process four times. Wash by filling each well with WB (Washing Buffer 300 µl). 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 µl of **TS** (TMB Substrate) to each well. Incubate for 5-30 minutes at room temperature.
9. Add 100 µl of **SS** (Stop Solution) to each well.
10. Determine the optical density within 30 minutes, using microplate **reader** set to 450 nm corrected with 570 nm or 630 nm.

TYPICAL DATA



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)

	Intra-assay Precision				Inter-assay Precision		
Sample Number	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 (ng/ml)	Detectable %	Mean of Detectable (ng/ml)
Serum	30	3.35-128.74	100	34.32

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**.