Human PDGF-AA ELISA Assay Kit

Catalog Number:
FDG31-K01 (1 x 96 wells)

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

v. 1.0 (19 AUG 24)

Eagle Biosciences, Inc.
20A Northwest Blvd., Suite 112, Nashua, NH 03063
Phone: 617-419-2019 Fax: 617-419-1110
www.EagleBio.com

INTENDED USE

The Eagle Biosciences Human PDGF-AA ELISA Assay Kit is intended for the quantitative measurement of PDGF-AA in human serum/plasma, urine, and more. The PDGF-AA 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 (Detect Antibody)	6 mL/bottle
SD (Sample Diluent)	12 mL/bottle
SH (Streptavidin-HRP)	12 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.

SAMPLE DILUTION

Samples such as serum and plasma require at least a 10-fold dilution into Sample Diluent. A suggested 10-fold dilution is 20 µL of sample + 180 µL of Sample Diluent.

REAGENT PREPARATION

Washing Buffer (1x) Preparation:

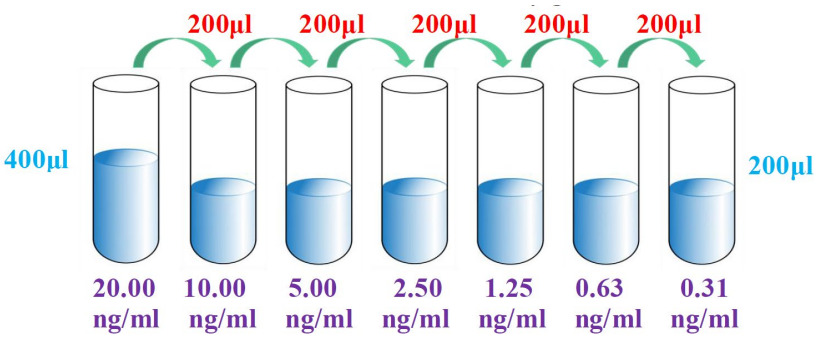
Pour entire contents (50 mL) of the Washing Buffer Concentrate (10x) into a clean 500 mL graduated cylinder. Bring to final volume of 500 mL with glass-distilled or deionized water. Transfer to a clean wash bottle and store at 2 to 25°C.

Standard Curve Preparation:

S1 to S7 and S0 are ready to use for serum and plasma.

For other sample types, prepare the standard curve with whatever buffer (SPB, Sample Prepared Buffer) is used to prepare the sample, such as cell culture supernatant, tissue grinding liquid, cell lysate, etc. For urine samples use AB (Assay Buffer) to prepare the standard curve.

The Human PDGF-AA Standard 200 pg/mL 40 µL + 360 µL SPB serves as the high standard (20 ng/mL). Pipette 200 µL of SPB into each tube. Use the high standard to produce a 1:1 dilution series. Mix each tube thoroughly before the next transfer. SPB serves as the zero standard (0 ng/mL).



ASSAY PROCEDURE

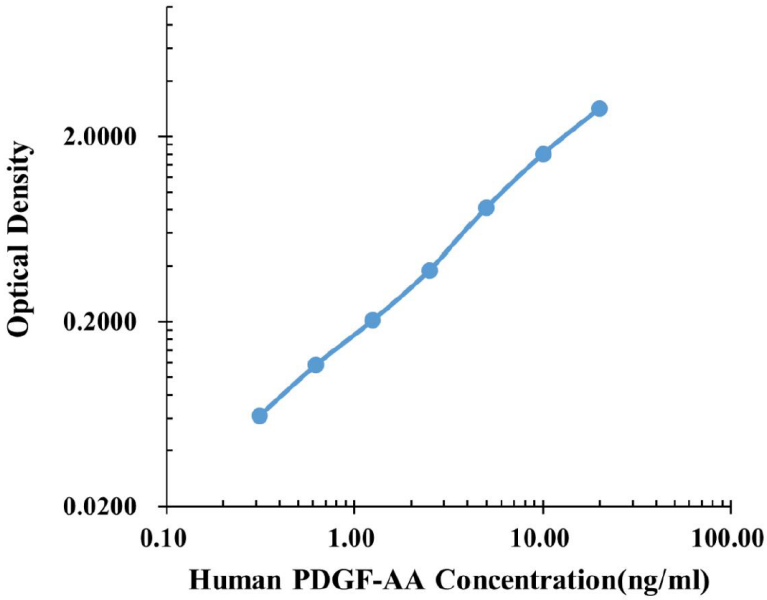
Bring all reagents and samples to room temperature before use.

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 µL of **AB (Assay Buffer)** to each well.
4. Add 50 µL or 10 uL of **Standard or Sample** per well. Ensure reagent addition is uninterrupted and completed withing 15 minutes.
5. Add 50 µL of **DA (Detect Antibody)** 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 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 **SH (Stretavidin-HRP)** to each well.
9. Cover with a new **SF (Sealer Film).** Incubate at room temperature (18 to 25°C) for 30 minutes on a microplate **shaker** set at 500 rpm.
10. Repeat aspiration/**wash** as in step 7.
11. Add 100 µL of **TS (TMB Substrate)** to each well. Incubate for 5-30 minutes at room temperature.
12. Add 100 µL of **SS (Stop Solution)** to each well.
13. Determine the optical density within 30 minutes, using a microplate **reader** set to 450 nm corrected with 570 nm or 630 nm.

TYPICAL DATA

ng/mL	O.D.		Average	Corrected
0.00	0.2311	0.2403	0.2357	
0.31	0.2942	0.3011	0.2977	0.0619
0.63	0.3271	0.3783	0.3527	0.1170
1.25	0.4328	0.4453	0.4391	0.2034
2.50	0.6121	0.6172	0.6147	0.3790
5.00	1.0300	1.0830	1.0565	0.8208
10.00	1.8060	1.8730	1.8395	1.6038
20.00	3.0540	3.0790	3.0665	2.8308

Human PDGF-AA Typical Standard



SENSITIVITY

The minimum detectable dose (MDD) of human PDGF-AA is typically less than 0.10 ng/mL (50 µL of sample volume) or 0.17 ng/mL (10 µL of sample volume).

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)	0.41	2.16	6.48	0.50	2.35	6.85
Standard Deviation	0.03	0.11	0.29	0.03	0.15	0.35
Coefficient of variation (%)	6.7	5.2	4.5	7.0	6.2	5.1

RECOVERY

The spike recovery was evaluated by spiking 3 levels of human PDGF-AA into healy human serum samples. The un-spiked serum was used as a blank in this experiment. The recovery ranged from 98% to 112% with an overall mean recovery of 106%.

LINEARITY

To assess the linearity of the assay, five samples were spiked with high concentration of PDGF0AA in human serum and diluted with Sample Diluent to produce samples with values within the dynamic range of the assay. The linearity ranged from 101% to 110% with an overall mean recovery of 108%.

SAMPLE VALUES

Serum/Plasma – Thirty samples from apparently healthy volunteers were evaluated for the presence of PDGF-AA 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	21.10 – 57.89	100%	33.58

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

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