

## ab300326 – Human PDGF B CatchPoint® SimpleStep ELISA® Kit

For the quantitative measurement of PDGF B in human serum, plasma (heparin), plasma (EDTA), plasma (citrate), and cell culture supernatant.  
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: [www.abcam.com/ab300326](http://www.abcam.com/ab300326)

**Storage and Stability:** Store kit at 2-8°C immediately upon receipt. Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Standard Preparation and Reagent preparation sections.

**Limitations:** All data, except Typical Standard Curve and Sensitivity were collected using the colorimetric version of this kit (ab300321).

### Materials Supplied

Item	Quantity	Storage Condition
Human PDGF B Capture Antibody 10X	600 µL	+4°C
Human PDGF B Detector Antibody 10X	600 µL	+4°C
Human PDGF B Lyophilized Recombinant Protein	2 Vials	+4°C
Antibody Diluent 5BI	6 mL	+4°C
Sample Diluent NS	12 mL	+4°C
Wash Buffer PT 10X	20 mL	+4°C
Stoplight Red Substrate Buffer	12 mL	+4°C
100X Stoplight Red Substrate	120 µL	+4°C
500X Hydrogen Peroxide (H <sub>2</sub> O <sub>2</sub> , 3%)	50 µL	+4°C
SimpleStep Pre-Coated Black 96-Well Microplate	96 Wells	+4°C
Plate Seal	1	+4°C

### Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

Fluorescence microplate reader Ex/Cutoff/Em 530/570/590 nm.

Deionized water.

Multi- and single-channel pipettes.

Tubes for standard dilution.

Plate shaker for all incubation steps.

Optional: Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).

### Reagent Preparation

Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells. The sample volumes below are sufficient for 48 wells (6 x 8-well strips); adjust volumes as needed for the number of strips in your experiment.

Prepare only as much reagent as is needed on the day of the experiment. Capture and Detector Antibodies have only been tested for stability in the provided 10X formulations

**1X Wash Buffer PT:** Prepare 1X Wash Buffer PT by diluting Wash Buffer PT 10X with deionized water. To make 50 mL 1X Wash Buffer PT combine 5 mL Wash Buffer PT 10X with 45 mL deionized water. Mix thoroughly and gently.

**Antibody Cocktail:** Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent 5BI. To make 3 mL of the Antibody Cocktail combine 300 µL 10X Capture Antibody and 300 µL 10X Detector Antibody with 2.4 mL Antibody Diluent 5BI. Mix thoroughly and gently.

**CatchPoint HRP Development Solution:** Just prior to use prepare CatchPoint HRP Development Solution by diluting the 100X Stoplight Red Substrate and the 500X Hydrogen Peroxide in Stoplight Red Substrate Buffer. For example, to make 6 mL of the CatchPoint HRP Development Solution combine 60 µL 100X Stoplight Red Substrate and 12 µL of 500X Hydrogen Peroxide with 5.928 mL Stoplight Red Substrate Buffer. Mix thoroughly and gently.

### Standard Preparation

Always prepare a fresh set of standards for every use. Discard working standard dilutions after use as they do not store well. The following section describes the preparation of a standard curve for duplicate measurements (recommended).

1. Reconstitute the PDGF B standard sample by adding the volume of Sample Diluent NS indicated on the protein vial label. Hold at room temperature for 10 minutes. Mix thoroughly and gently. This is the 8,000 pg/mL **Stock Standard** Solution.
2. Label fourteen tubes, Standards 1– 14.
3. Add 200 µL of Sample Diluent NS into tube number 1 and 150 µL of Sample Diluent NS into numbers 2-14.
4. Use the **Stock Standard** to prepare the following dilution series. Standard #14 contains no protein and is the Blank control:

Standard #	Dilution Sample	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	<b>Stock Standard</b>	200	200	8,000	4,000
2	Standard#1	150	150	4,000	2,000
3	Standard#2	150	150	2,000	1,000
4	Standard#3	150	150	1,000	500
5	Standard#4	150	150	500	250
6	Standard#5	150	150	250	125
7	Standard#6	150	150	125	62.5
8	Standard#7	150	150	62.5	31.25
9	Standard#8	150	150	31.25	15.63
10	Standard#9	150	150	15.63	7.81
11	Standard#10	150	150	7.81	3.91
12	Standard#11	150	150	3.91	1.95
14	Blank Control	0	150	N/A	N/A

## Sample Preparation

Typical Sample Dynamic Range	
Sample Type	Range
Serum	≤12.5%
Plasma - Heparin	≤12.5%
Plasma - EDTA	≤12.5%
Plasma - Citrate	3.13 – 25%
PBMC Stimulated Culture Supernatant	3.13 – 50%
PBMC Unstimulated Culture Supernatant	6.25 – 50%

**Serum** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2,000 x g for 10 minutes and collect serum. Dilute samples at least 1:8 into Sample Diluent NS and assay. Store un-diluted serum at -20°C or below. Avoid repeated freeze-thaw cycles.

**Plasma** Collect plasma using citrate, EDTA or heparin. Centrifuge samples at 2,000 x g for 10 minutes. Dilute plasma (EDTA and heparin) samples at least 1:8 into Sample Diluent NS and assay. Dilute plasma (citrate) samples at least 1:4 into Sample Diluent NS and assay. Store un-diluted plasma samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

**Cell Culture Supernatants** Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Collect supernatants. Dilute samples at least 1:2 into Sample Diluent NS and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

## Plate Preparation

The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.

Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4°C.

For each assay performed, a minimum of two wells must be used as the zero control.

For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

Differences in well absorbance or "edge effects" have not been observed with this assay.

## Assay Procedure

Equilibrate all materials and prepared reagents to room temperature prior to use.

We recommend that you assay all standards, controls and samples in duplicate

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.
3. Add 50 µL of all sample or standard to appropriate wells.
4. Add 50 µL of the Antibody Cocktail to each well.

5. Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm.
6. Wash each well with 3 x 350 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 350 µL 1X Wash Buffer PT into each well. Wash Buffer PT should remain in wells for at least 10 seconds. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and tap gently against clean paper towels to remove excess liquid.
7. Add 100 µL of prepared CatchPoint HRP Development Solution to each well and incubate for 10 minutes in the dark on a plate shaker set to 400 rpm. Further optimization of incubation time vs signal strength can be performed if needed.
8. Record the fluorescence at Ex/Cutoff/Em 530/570/590 nm. If using a Molecular Devices' plate reader supported by SoftMax® Pro software, a preconfigured protocol for these CatchPoint SimpleStep ELISA Kits is available with all the protocol and analysis settings at [www.softmaxpro.org](http://www.softmaxpro.org).
9. Analyze the data as described below.

Mode	Fluorescence
Instrument settings:	Endpoint
Excitation:	530 nm
Cutoff:	570 nm
Emission:	590 nm
Sensitivity:	6 flashes/read or 200ms
PMT:	Auto
Auto calibrate:	On
Read:	Top
Read Height:	1*

**Note** For microplate readers with Pre-Read Optimization option, the Read Height as well as Microplate Optimization is recommended before the first read.

**Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:**

[www.abcam.com/protocols/the-complete-elisa-guide](http://www.abcam.com/protocols/the-complete-elisa-guide)

**For technical support contact information, visit:** [www.abcam.com/contactus](http://www.abcam.com/contactus)

[www.abcam.com/contactus](http://www.abcam.com/contactus)

[www.abcam.cn/contactus](http://www.abcam.cn/contactus) (China)

[www.abcam.co.jp/contactus](http://www.abcam.co.jp/contactus) (Japan)

Copyright © 2022 Abcam, All Rights Reserved. All information / detail is correct at time of going to print.  
Version 1d | 2022-08-22

# ab300326 – Human PDGF B CatchPoint® SimpleStep ELISA® Kit

## Additional information

### ASSAY SPECIFICITY

This kit is designed for the quantification of human PDGF B.

The standard protein in this kit is a full length human PDGF B.

This kit detects PDGF B monomers and homodimers.

Native signal was detected in serum, plasma (heparin), plasma (EDTA), plasma (citrate), and cell culture supernatant.

Spiked protein experiments were used to validate serum, plasma (heparin), and plasma (EDTA) sample types.

Saliva, urine, milk, CSF, cell extract, and tissue extract samples have not been tested with this kit.

### CROSS REACTIVITY

500 pg/mL of recombinant human PDGF AB was tested for cross reactivity. 0.8% cross reactivity was observed.

### INTERFERENCE

250 pg/mL of recombinant human PDGF AB was tested for interference with 250 pg/mL of recombinant human PDGF B. No interference was observed.

### SPECIES REACTIVITY

500 pg/mL of recombinant mouse PDGF B was tested for reactivity. 44% reactivity was observed.

Other species reactivity was determined by measuring serum samples of various species, interpolating the protein concentrations from the human standard curve, and expressing the dilution factor-corrected interpolated concentrations in pg per mL of neat sera.

Species Serum	Dilution Factor	Interpolated Values
Human	1:8	91.55
Primate	1:100	6,178.43
COW	1:8	Not Detectable
Mouse	1:100	5,703.00
Rat	1:8	1,427.54

Other species reactivity not determined.

### CALCULATION

- Preconfigured protocols are available when using SoftMax Pro software from Molecular Devices
- Calculate the average fluorescence value for the blank control (zero) standards. Subtract the average blank control standard fluorescence value from all other fluorescence values.
- Create a standard curve by plotting the average blank control subtracted fluorescence value for each standard concentration (y-axis) against the target protein concentration

(x-axis) of the standard. Use graphing software to draw the best smooth curve through these points to construct the standard curve.

- Note: Most fluorescence reader software or graphing software will plot these values and fit a curve to the data. A four-parameter curve fit (4PL) is often the best choice; however, other algorithms (e.g. linear, semi-log, log/log, 4-parameter logistic) can also be tested to determine if it provides a better curve fit to the standard values.
- Determine the concentration of the target protein in the sample by interpolating the blank control subtracted fluorescence values against the standard curve. Multiply the resulting value by the appropriate sample dilution factor, if used, to obtain the concentration of target protein in the sample.
- Samples generating fluorescence values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at fluorescence values less than that of the lowest standard should be retested in a less dilute form.

### TYPICAL DATA

Typical standard curve – data provided for demonstration purposes only. A new standard curve must be generated for each assay performed

Standard Curve Measurements			
Concentration (pg/mL)	RFU		Mean RFU
	1	2	
0	403715	403715	403715
1.95	1187220	1329338	1258279
3.91	1724311	1846872	1785592
7.81	2073706	2323140	2198423
15.63	4325040	4652011	4488526
31.25	6551504	6272120	6411812
62.5	14194205	15438843	14816524
125	46680924	48491860	47586392
250	57218312	61924236	59571274
500	138188880	151983104	145085992
1,000	218917344	206691200	212804272
2,000	319211360	316161536	317686448
4,000	416514944	413974848	415244896

Table 1. Example of human PDGF B standard curve in Sample Diluent NS. The PDGF B standard curve was prepared as described in the Standard Preparation section. Raw data generated on SpectraMax M4 Multi-Mode Microplate Reader is shown in the table. Background-subtracted data values (mean +/- SD) are graphed.

## TYPICAL SAMPLE VALUES

### Sensitivity:

The calculated minimal detectable dose (MDD) is 1.57 pg/mL. The MDD was determined by calculating the mean of zero standard replicates (n=22) and adding 2 standard deviations then extrapolating the corresponding concentration.

### Recovery

Three concentrations of PDGF B were spiked in duplicate to the indicated biological matrix to evaluate signal recovery in the working range of the assay.

Sample Type	Average % Recovery	Range (%)
12.5 % Serum	99	95 - 103
12.5 % Plasma - EDTA	118	110 - 124
12.5 % Plasma - Citrate	86	81 - 89
12.5 % Plasma - Heparin	105	93 - 116
15 % Stim. PBMC Culture Supernatant*	108	80 - 122
15% Unstim. PBMC Culture Supernatant	116	97 - 129

\*Media is RPMI 1640 containing 10% fetal calf serum.

### Linearity of Dilution

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

Native PDGF B was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS.

Dilution Factor	Interpolated value	12.5% Human Serum	12.5% Human Plasma (EDTA)	25% Human Plasma (Citrate)	12.5% Human Plasma (Heparin)	50% Stim. PBMC Sup.
Undiluted	pg/mL	10.40	21.82	68.05	20.62	167.32
	% Expected value	100	100	100	100	100
2	pg/mL	ND	11.36	35.96	12.09	88.82
	% Expected value		104	106	117	106
4	pg/mL	ND	ND	18.56	ND	39.50
	% Expected value			109		94
8	pg/mL	ND	ND	9.97	ND	21.66
	% Expected value			117		104
16	pg/mL	ND	ND	ND	ND	11.50
	% Expected value					110

ND – Not Detected – below product dynamic range

Recombinant PDGF B was spiked in in the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS.

Dilution Factor	Interpolated value	12.5% Human Serum	12.5% Human Plasma (EDTA)	12.5% Human Plasma (Heparin)
Undiluted	pg/mL	60.50	122.26	133.61
	% Expected value	100	100	100
2	pg/mL	36.18	71.60	67.82
	% Expected value	120	117	102
4	pg/mL	16.50	39.13	39.89
	% Expected value	109	125	119
8	pg/mL	7.85	18.38	17.65
	% Expected value	105	120	106
16	pg/mL	ND	8.92	10.02
	% Expected value		117	120

ND – Not Detected – below product dynamic range

### Precision

Mean coefficient of variations of interpolated values of PDGF B from three concentrations of human plasma (citrate) within the working range of the assay.

	Intra-assay	Inter-assay
N=	8	3
CV (%)	4.7	6.0

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:

[www.abcam.com/protocols/the-complete-elisa-guide](http://www.abcam.com/protocols/the-complete-elisa-guide)

For technical support contact information, visit:

[www.abcam.com/contactus](http://www.abcam.com/contactus)

[www.abcam.cn/contactus](http://www.abcam.cn/contactus) (China)

[www.abcam.co.jp/contactus](http://www.abcam.co.jp/contactus) (Japan)

Copyright © 2022 Abcam, All Rights Reserved. All information / detail is correct at time of going to print. Version 1d | 2022-08-22