ab300325 – Human Factor IX/PTC CatchPoint® SimpleStep ELISA® Kit

For the quantitative measurement of Factor IX/PTC in human serum, plasma (heparin), plasma (EDTA), plasma (citrate).
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab300325

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 (ab300307).

Materials Supplied

Item	Quantity	Storage Condition
Human Factor IX/PTC Capture Antibody 10X	600 µL	+4°C
Human Factor IX/PTC Detector Antibody 10X	600 µL	+4°C
Human Factor IX/PTC Lyophilized Recombinant Protein	2 Vials	+4°C
Antibody Diluent 4BI	6 mL	+4°C
Sample Diluent NS	50 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 (H2O2, 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 4BI. 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 4BI. 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 Factor IX/PTC 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 100,000 pg/mL **Stock Standard** Solution.
2. Label eight tubes, Standards 1– 8.
3. Add 280 µL of Sample Diluent NS into tube number 1 and 150 µL of Sample Diluent NS into numbers 2-8.
4. Use the **Stock Standard** to prepare the following dilution series. Standard #8 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	**Stock Standard**	280	120	100,000	30,000
2	Standard#1	150	150	30,000	15,000
3	Standard#2	150	150	15,000	7,500
4	Standard#3	150	150	7,500	3,250
5	Standard#4	150	150	3,250	1,875
6	Standard#5	150	150	1,875	937.5
7	Standard#6	150	150	937.5	468.75
8	Standard#7	150	150	468.75	234.38
9	Standard#8	150	150	234.38	117.19
10	Standard#9	150	150	117.19	58.59

11	Standard#10	150	150	58.59	29.30
12	Standard#11	150	150	29.30	14.65
13	Blank Control	0	150	N/A	N/A

Sample Preparation

Typical Sample Dynamic Range	
Sample Type	**Range**
Serum	0.019 – 0.3%
Plasma - Heparin	0.0094 – 0.15%
Plasma - EDTA	0.0094 – 0.15%
Plasma - Citrate	0.019 – 0.15%

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 1:400 into Sample Diluent NS and assay. Store un-diluted serum at -20ºC 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 samples at least 1:700 into Sample Diluent NS and assay. Store un-diluted plasma samples at -20ºC or below for up to 3 months. 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 4ºC.
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 4ºC 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.
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

For technical support contact information, visit:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
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Additional information

ASSAY SPECIFICITY

This kit is designed for the quantification of human Factor IX/PTC.

The standard protein in this kit is full length human Factor IX/PTC.

Native signal was detected in serum, plasma (heparin), plasma (EDTA), plasma (citrate).

Cell culture supernatant, saliva, urine, milk, CSF, cell extract, and tissue extract samples have not been tested with this kit.

SPECIES REACTIVITY

Other species reactivity was determined by measuring 0.05% serum samples of various species, interpolating the protein concentrations from the human standard curve, and expressing the interpolated concentrations as a percentage of the protein concentration in human serum assayed at the same dilution.

Reactivity < 3% was determined for the following species: Mouse, Rat, Cow

This kit is 112% reactive with monkey samples.

Other species reactivity not determined.

CALCULATION

- Calculate the average absorbance value for the blank control (zero) standards. Subtract the average blank control standard absorbance value from all other absorbance values.
- Create a standard curve by plotting the average blank control subtracted absorbance 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.

  Δ Note: Most microplate 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 absorbance 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 absorbance values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at an absorbance 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	
14.65	362,013	337,277	349,645
29.30	529,427	426,772	478,099
58.59	1,043,509	708,798	876,153
117.19	1,948,607	1,961,736	1,955,171
234.38	3,256,440	2,528,849	2,892,644
468.75	5,597,663	3,348,120	4,472,891
937.5	8,831,455	8,106,405	8,468,930
1,875	21,276,144	23,333,086	22,304,615
3,750	33,662,880	32,601,554	33,132,217
7,500	64,491,672	73,506,356	68,999,014
15,000	118,819,436	141,559,884	130,189,660
30,000	204,296,076	216,181,196	210,238,636

Table 1. Example of human Factor IX/PTC standard curve in Sample Diluent NS. The Factor IX/PTC 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 15.9 pg/mL. The MDD was determined by calculating the mean of zero standard replicates (n=24) and adding 2 standard deviations then extrapolating the corresponding concentration.

Recovery

Three concentrations of Factor IX/PTC 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 (%)
0.1% Serum	105	92 - 113
0.05% Plasma - Citrate	116	111 - 119
0.05% Plasma - EDTA	106	95 - 115
0.05% Plasma - Heparin	112	103 - 118

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 Factor IX/PTC 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	0.15% Human Serum	0.075% Human Plasma (Citrate)	0.075% Human Plasma (EDTA)	0.15% Human Plasma (Heparin)
Undiluted	pg/mL	2414.56	4043.74	3681.26	5404.24
	% Expected value	100	100	100	100
2	pg/mL	1226.11	1898.81	1493.09	2657.96
	% Expected value	102	94	81	98
4	pg/mL	632.54	967.36	873.97	1258.03
	% Expected value	105	96	95	93
8	pg/mL	338.86	475.44	446.06	664.07
	% Expected value	112	94	97	98
16	pg/mL	175.87	286.12	251.03	403.37
	% Expected value	117	113	109	119

Precision

Mean coefficient of variations of interpolated values of Factor IX/PTC from one concentration of serum within the working range of the assay.

	Intra-assay	Inter-assay
N=	**8**	**3**
CV (%)	**7.4**	**10.1**

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
www.abcam.com/protocols/the-complete-elisa-guide

For technical support contact information, visit:
www.abcam.com/contactus
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