ab300318 – Human Factor B SimpleStep ELISA® Kit

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

For overview, typical data and additional information please visit: www.abcam.com/ab300318

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.

Materials Supplied

Item	Quantity	Storage Condition
Human Factor B Capture Antibody 10X	600 µL	+4°C
Human Factor B Detector Antibody 10X	600 µL	+4°C
Human Factor B Lyophilized Recombinant Protein	2 Vials	+4°C
Antibody Diluent 4BR	6 mL	+4°C
Sample Diluent NS	2 x 50 mL	+4°C
Wash Buffer PT 10X	20 mL	+4°C
TMB Development Solution	12 mL	+4°C
Stop Solution	12 mL	+4°C
SimpleStep Pre-Coated 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:

Microplate reader capable of measuring absorbance at 450 or 600 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 4BR. 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 4BR. 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 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 48 ng/mL **Stock Standard** Solution.
2. Label eight tubes, Standards 1– 8.
3. Add 380 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**	29	380	48,000	3,400
2	Standard#1	150	150	3,400	1,700
3	Standard#2	150	150	1,700	850
4	Standard#3	150	150	850	425
5	Standard#4	150	150	425	212.5
6	Standard#5	150	150	212.5	106.25
7	Standard#6	150	150	106.25	53.13
8	Blank Control	0	150	N/A	N/A

Sample Preparation

Typical Sample Dynamic Range	
Sample Type	**Range**
Serum	1:100,000 - 1:1,600,000
Plasma - EDTA	1:100,000 - 1:1,600,000
Plasma - Citrate	1:100,000 - 1:1,600,000
Plasma - Heparin	1:100,000 - 1:1,600,000
HepG2 Cell Culture Supernatant	1:8 - 1:128
Saliva	1:16 - 1:256
Milk	1:800 - 1:12,800
Urine	<50%

Note: Due to the high dilutions required for serum and plasma samples, we recommend initially diluting your samples in 1X Wash Buffer before the final dilution in Sample Diluent NS. The following table has a suggested dilution scheme.

Tube #	Sample to Dilute	Volume of sample (µL)	Volume of 1X Wash Buffer (µL)	Volume of Sample Diluent NS (µL)	Starting Conc.	Final Conc.
1	Neat serum	5	495	0	Neat	1:100
2	Tube #1	5	495	0	1:100	1:10,000
3	Tube #2	50	0	450	1:10,000	1:100,000

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 1:100,000 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 1:100,000 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.

Cell Culture Supernatants Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Collect supernatants and assay. Or dilute samples at least 1:8 into Sample Diluent NS and assay. Store un-diluted samples at -20ºC or below. Avoid repeated freeze-thaw cycles.

Urine Centrifuge urine at 2,000 x g for 10 minutes to remove debris. Dilute samples at least 1:2 into Sample Diluent NS and assay. Store un-diluted urine samples at -20ºC or below. Avoid repeated freeze-thaw cycles.

Saliva Centrifuge saliva at 800 x g for 10 minutes to remove debris. Collect supernatants and dilute samples at least 1:16 into Sample Diluent NS and assay. Store un-diluted samples at -20ºC or below. Avoid repeated freeze-thaw cycles.

Milk De-fat milk samples as follows. Centrifuge milk samples at 500 x g for 15 minutes at 4ºC and collect the aqueous fraction using syringe attached to needle. Centrifuge the aqueous fraction at 3,000 x g for 15 minutes at 4ºC and collect the final aqueous fraction (de-fatted milk) using syringe attached to needle. Dilute the de-fatted milk samples at least 1:800 into Sample Diluent NS and assay. Store un-diluted de-fatted milk at -20ºC 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 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 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 TMB Development Solution to each well and incubate for 10 minutes in the dark on a plate shaker set to 400 rpm.
   Given variability in laboratory environmental conditions, optimal incubation time may vary between 5 and 20 minutes.
   Note: The addition of Stop Solution will change the color from blue to yellow and enhance the signal intensity about 3X. To avoid signal saturation, proceed to the next step before the high concentration of the standard reaches a blue color of O.D.600 equal to 1.0.
8. Add 100 µL of Stop Solution to each well. Shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm. This is an endpoint reading.
9. Alternative to 13.7 – 13.8: Instead of the endpoint reading at 450 nm, record the development of TMB Substrate kinetically. Immediately after addition of TMB Development Solution begin recording the blue color development with elapsed time in the microplate reader prepared with the following settings:

Mode	Kinetic
Wavelength:	600 nm
Time:	up to 20 min
Interval:	20 sec - 1 min
Shaking:	Shake between readings

   Note that an endpoint reading can also be recorded at the completion of the kinetic read by adding 100 µL Stop Solution to each well and recording the OD at 450 nm.

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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Additional information

ASSAY SPECIFICITY
This kit is designed for the quantification of human Factor B.

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

This kit detects full length human Complement Factor B but does not detect Human Factor B fragments Ba or Bb.

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

Spiked protein experiments were used to validate urine.

Cell extract and tissue extract samples have not been tested with this kit.

CROSS REACTIVITY
3,400 pg/mL of recombinant human Factor B Ba fragment, 3400 pg/mL of recombinant human Factor B Bb fragment, and 3,400 pg/mL of recombinant human Factor D (Adipsin) were tested for cross reactivity. No cross reactivity was observed.

INTERFERENCE
50,000 pg/mL of recombinant human Adipsin (Complement Factor D) was tested for interference with 850 pg/mL of recombinant Factor B. No interference was observed.

SPECIES REACTIVITY
Other species reactivity was determined by measuring 1:100,000 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.

No signal was observed in 1:100,000 serum samples from the following species: Monkey, Mouse, Rat, Cow

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)	O.D 450 nm		Mean O.D
	1	2	
0	0.064	0.066	0.065
53.1	0.111	0.109	0.110
106.3	0.157	0.156	0.157
212.5	0.243	0.251	0.247
425.0	0.427	0.431	0.429
850	0.741	0.800	0.771
1,700	1.415	1.495	1.455
3,400	2.953	2.914	2.934

Table 1. Example of human Factor B standard curve in Sample Diluent NS. The Factor B standard curve was prepared as described in the Standard Preparation section. The table shows raw data values.

TYPICAL SAMPLE VALUES
Sensitivity:
The calculated minimal detectable dose (MDD) is 18.2 pg/mL. The MDD was determined by calculating the mean of zero standard replicates (n=16) and adding 2 standard deviations then extrapolating the corresponding concentration.

Recovery
3 concentrations of Factor 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 (%)
1:200,000 Serum	91	88 - 94
1:200,000 Plasma - EDTA	99	92 - 106
1:200,000 Plasma - Citrate	105	103 - 110
1:200,000 Plasma - Heparin	96	94 - 98
1:20 HepG2 Cell Culture Supernatant	91	86 - 100
1:20 Saliva	85	82 - 88
1:2,000 Milk	94	90 - 100
50% Urine	82	82 - 83

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 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	1:100,000 Human Serum	1:100,000 Human Plasma (Citrate)	1:100,000 Human Plasma (EDTA)	1:100,000 Human Plasma (Heparin)
Undiluted	pg/mL	2,234.5	2,047.1	2,231.7	1,638.4
	% Expected value	100	100	100	100
2	pg/mL	1,056.2	977.9	952.8	717.7
	% Expected value	95	96	85	88
4	pg/mL	453.4	481.5	406.5	393.9
	% Expected value	81	94	73	96
8	pg/mL	237.3	211.3	257.4	205.5
	% Expected value	85	83	92	100
16	pg/mL	144.7	118.6	138.1	98.2
	% Expected value	104	93	99	96

Dilution Factor	Interpolated value	1:8 HepG2 Cell Culture Supernatant	1:16 Human Saliva	1:800 Human Milk
Undiluted	pg/mL	2,512.5	1,063.9	2,210.6
	% Expected value	100	100	100
2	pg/mL	1,422.6	532.2	1,153.0
	% Expected value	113	100	104
4	pg/mL	636.0	281.4	602.8
	% Expected value	101	106	109
8	pg/mL	352.1	152.9	309.2
	% Expected value	112	115	112
16	pg/mL	141.5	56.2	132.8
	% Expected value	90	84	96

Recombinant Factor 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	50% Human Urine
Undiluted	pg/mL	1,535.9
	% Expected value	100
2	pg/mL	776.1
	% Expected value	101
4	pg/mL	426.6
	% Expected value	111
8	pg/mL	220.6
	% Expected value	115
16	pg/mL	109.4
	% Expected value	114

Precision

Mean coefficient of variations of interpolated values of Factor B from a single concentration of human serum within the working range of the assay.

	Intra-assay	Inter-assay
N=	8	3
CV (%)	4.12	5.72

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