ab230936 – Human IGFBP4 SimpleStep ELISA® Kit

For the quantitative measurement of IGFBP4 in human serum, plasma (citrate), plasma (EDTA), plasma (heparin), 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/ab230936

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 1 x 96 tests	Storage Condition
Human IGFBP4 Capture Antibody 10X	600 µL	+4°C
Human IGFBP4 Detector Antibody 10X	600 µL	+4°C
Human IGFBP4 Lyophilized Recombinant Protein	2 Vials	+4°C
Antibody Diluent CPI2	6 mL	+4°C
Sample Diluent NS	12 mL	+4°C
Wash Buffer PT 10X	2 x 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

Sample Diluent NS is provided but not necessary for this product.

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 100 mL 1X Wash Buffer PT combine 10 mL Wash Buffer PT 10X with 90 mL deionized water. Mix thoroughly and gently.

Antibody Cocktail: Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent CPI2. 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 CPI2. 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. **IMPORTANT:** If the protein standard vial has a volume identified on the label, reconstitute the IGFBP4 standard sample by adding that volume of 1X Wash Buffer PT indicated on the label. Alternatively, if the vial has a mass identified, reconstitute the IGFBP4 standard sample standard by adding 500 µL 1X Wash Buffer PT. Hold at room temperature for 10 minutes and mix gently. This is the 50 ng/mL **Stock Standard** Solution.
2. Label eight tubes, Standards 1– 8.
3. Add 270 µL of 1X Wash Buffer PT into tube number 1 and 150 µL of 1X Wash Buffer PT 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**	30	270	50,000	5,000
2	Standard#1	150	150	5,000	2,500
3	Standard#2	150	150	2,500	1,250
4	Standard#3	150	150	1,250	625
5	Standard#4	150	150	625	312.5
6	Standard#5	150	150	312.5	156.25
7	Standard#6	150	150	156.25	78.13
8	Blank Control	0	150	0	0

Sample Preparation

Typical Sample Dynamic Range	
Sample Type	**Range**
Serum	1:3,200 - 1:200
Plasma – Citrate	1:1,600 - 1:100
Plasma – EDTA	1:1,600 - 1:100
Plasma – Heparin	1:1,600 - 1:100
A549 Cell Culture Supernatant	1:1,600 - 1:100

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 1:200 into 1X Wash Buffer PT 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 samples at least 1:100 into 1X Wash Buffer PT 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 and assay. Or dilute samples at least 1:100 into 1X Wash Buffer PT 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 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 7 – 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

Version 4a | 2025-09-09

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Additional information

ASSAY SPECIFICITY

This kit is designed for the quantification of human IGFBP4.

The standard protein in this kit is full-length mature human IGFBP4.

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

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

INTERFERENCE

1,250 pg/mL of recombinant human IGFBP4 was assayed in the presence of recombinant human IGF1 or recombinant human IGF2. The % decrease in human IGFBP4 signal is show below.

Recombinant Human IGF1 (pg/mL)	% Decrease in IGFBP4 Signal
50,000	12%
5,000	7%
1,250	2%

Recombinant Human IGF2 (pg/mL)	% Decrease in IGFBP4 Signal
50,000	21%
5,000	8%
1,250	0%

SPECIES REACTIVITY

Other species reactivity was determined by measuring 1:400 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.

Interpolated values were below the detectable range of the assay for the following species: 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 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	0.087	0.089	0.088
78.13	0.132	0.130	0.131
156.25	0.215	0.217	0.216
312.5	0.321	0.315	0.318
625	0.587	0.545	0.566
1,250	0.980	0.980	0.980
2,500	1.989	1.881	1.935
5,000	3.543	3.531	3.537

Table 1. Example of human IGFBP4 standard curve in 1X Wash Buffer PT. The IGFBP4 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 32.6 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

Three concentrations of IGFBP4 were spiked into the indicated biological matrix to evaluate signal recovery in the working range of the assay.

Sample Type	Average % Recovery	Range (%)
1:1,000 Serum	103	102 - 104
1:1,000 Plasma – Citrate	112	110 - 116
1:500 Plasma – EDTA	93	89 - 97
1:500 Plasma – Heparin	106	104 - 109
1:500 A549 Supernatant	103	96 - 113

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 IGFBP4 was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in 1X Wash Buffer PT.

Dilution Factor	Interpolated value	1:200 Human Serum	1:100 Human Plasma (Citrate)	1:100 Human Plasma (EDTA)	1:100 Human Plasma (Heparin)	1:100 A549 Sup
Undiluted	pg/mL	2,503.3	5,045.8	3,734.7	3,039.6	4,211.5
	% Expected value	100	100	100	100	100
2	pg/mL	1,204.4	2,302.3	1,738.6	1,440.4	2,122.4
	% Expected value	96	91	93	95	101
4	pg/mL	599.9	1,119.9	845.4	708.9	1,051.1
	% Expected value	96	89	91	93	100
8	pg/mL	329.5	576.8	441.8	375.9	548.5
	% Expected value	105	91	95	99	104
16	pg/mL	186.7	258.8	188.2	152.8	262.5
	% Expected value	119	82	81	80	100

Precision

Mean coefficient of variations of interpolated values of IGFBP4 from a single concentration of human serum within the working range of the assay.

	Intra-assay	Inter-assay
N=	8	3
CV (%)	5.5	11.7

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
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Version 4a | 2025-09-09