ab327895 – Human IGFBP1 SimpleStep ELISA® Kit - Chemiluminescent

For the quantitative measurement of IGFBP1 in human serum, plasma (heparin), plasma (EDTA), plasma (citrate), cell culture supernatant, urine, milk, cell extract, and tissue extract.
For research use only - not intended for diagnostic use.
Patent pending.

For overview, typical data and additional information please visit: www.abcam.com/ab327895

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 (ab233618).

Materials Supplied

Item	Quantity 1 x 96 tests	Quantity 10 x 96 tests	Storage Condition
Human IGFBP1 Capture Antibody 10X	600 µL	10 x 600 µL	+4°C
Human IGFBP1 Detector Antibody 10X	600 µL	10 x 600 µL	+4°C
Human IGFBP1 Lyophilized Recombinant Protein	2 Vials	10 x 2 Vials	+4°C
Antibody Diluent 4BI	6 mL	10 x 6 mL	+4°C
Cell Extraction Buffer PTR 5X	10 mL	2 x 50 mL	+4°C
Cell Extraction Enhancer Solution 50X	1 mL	10 x 1 mL	+4°C
Sample Diluent NS	50 mL	2 x 250 mL	+4°C
Wash Buffer PT 10X	20 mL	200 mL	+4°C
ChemiHRP Reagent A	3 mL	50 mL	+4°C
ChemiHRP Reagent B	3 mL	50 mL	+4°C
SimpleStep Pre-Coated Black 96-Well Microplate	1 x 96 Wells	10 x 96 Wells	+4°C
Plate Seal	1	10	+4°C

Materials Required, Not Supplied

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

Luminometer capable of reading strip plates with the following settings: 0.5-1 second/well read time; summation mode (all wavelengths).
Method for determining protein concentration (BCA assay recommended).
Deionized water.
Multi- and single-channel pipettes.
Tubes for standard dilution.
Orbital microplate shaker for all incubation steps: capable of 750 rpm shaking speed.
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.

The provided Cell Extraction Enhancer Solution 50X may precipitate when stored at + 4°C. To dissolve, warm briefly at + 37°C and mix gently. The Cell Extraction Enhancer Solution 50X can be stored at room temperature to avoid precipitation.

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.

1X Cell Extraction Buffer PTR (For cell and tissue extracts only): Prepare 1X Cell Extraction Buffer PTR by diluting Cell Extraction Buffer PTR 5X and 50X Cell Extraction Enhancer Solution to 1X with deionized water. To make 10 mL 1X Cell Extraction Buffer PTR combine 7.8 mL deionized water, 2 mL Cell Extraction Buffer PTR 5X and 200 µL Cell Extraction Enhancer Solution 50X. Mix thoroughly and gently. If required protease inhibitors can be added.

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.

Lumi HRP Development Solution: Just prior to use, prepare Lumi HRP Development Solution by mixing equal volume of the ChemiHRP Reagent A and the ChemiHRP Reagent B. To make 3 mL of the Lumi HRP Development Solution combine 1.5 mL of ChemiHRP Reagent A and 1.5 mL of ChemiHRP Reagent B. Mix thoroughly and gently by inversion or slow pipetting (Avoid shaking or vortexing). Protect the prepared solution from light until use.

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 **IGFBP1** protein standard by adding the volume indicated on the protein vial label. For **serum, plasma, milk, and cell culture supernatant samples measurements**, use Sample Diluent NS. For **cell and tissue homogenate extract samples measurements**, use 1X Cell Extraction Buffer PTR. Hold at room temperature for 10 minutes and mix thoroughly and gently. This is the 10,800 pg/mL **Stock Standard** Solution.
1. Label nine tubes, Standards 1– 9.
2. Use the same Sample Diluent as used to resuspend the Stock Standard to prepare the standard curve.
3. Add 170 µL of Sample Diluent into tube number 1 and 150 µL of Sample Diluent into numbers 2-9.
4. Use the **Stock Standard** to prepare the following dilution series. Standard #9 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**	90	170	10,800	4,000
2	Standard#1	75	150	4,000	1,333
3	Standard#2	75	150	1,333	444.4
4	Standard#3	75	150	444.4	148.2
5	Standard#4	75	150	148.2	49.38
6	Standard#5	75	150	49.38	16.46
7	Standard#6	75	150	16.46	5.49
8	Standard#7	75	150	5.49	1.83
9	Blank Control	0	150	0	0

Sample Preparation

Typical Sample Dynamic Range	
Sample Type	**Range**
Serum	1:640 – 1:40
Plasma – Citrate	1:640 – 1:20
Plasma – EDTA	1:640 – 1:40
Plasma – Heparin	1:640 – 1:20
Urine*	1:128 – 1:8
Milk	1:640 – 1:20
A549 Supernatant	6.3 – 100%
HepG2 Supernatant	1:6,400 – 1:200
HepG2 Cell Extract	0.1 – 4 µg/ml
U-87 MG Cell Extract	31.3 – 500 µg/ml
Liver Tissue Homogenate Extract	15.6 – 500 µg/ml

*This sample type is validated by adding to the matrix human IGFBP1 standard protein to mimic native signal.

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 1:40 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 1:20 (citrate, heparin)or 1:40 (EDTA) 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.

Urine Centrifuge urine at 2,000 x g for 10 minutes to remove debris. Dilute samples at least 1:8 into Sample Diluent NS and assay. Store un-diluted urine 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 samples at least 1:20 into Sample Diluent NS and assay. Store un-diluted de-fatted milk at -20ºC or below. 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 into Sample Diluent NS and assay. Store un-diluted samples at -20ºC or below. Avoid repeated freeze-thaw cycles. Store un-diluted samples at -20ºC or below. Avoid repeated freeze-thaw cycles.

Preparation of extracts from cell pellets Collect non-adherent cells by centrifugation or scrape to collect adherent cells from the culture flask. Typical centrifugation conditions for cells are 500 x g for 5 minutes at 4ºC. Rinse cells twice with PBS. Solubilize pellet at $2x10^7$ cell/mL in chilled 1X Cell Extraction Buffer PTR. Incubate on ice for 20 minutes. Centrifuge at 18,000 x g for 20 minutes at 4ºC. Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and store at -80ºC. The sample protein concentration in the extract may be quantified using a protein assay. Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

Preparation of extracts from adherent cells by direct lysis (alternative protocol) Remove growth media and rinse adherent cells 2 times in PBS. Solubilize the cells by addition of chilled 1X Cell Extraction Buffer PTR directly to the plate (use 750 µL - 1.5 mL 1X Cell Extraction Buffer PTR per confluent 15 cm diameter plate). Scrape the cells into a microfuge tube and incubate the lysate on ice for 15 minutes. Centrifuge at 18,000 x g for 20 minutes at 4ºC. Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and store at -80ºC. The sample protein concentration in the extract may be quantified using a protein assay. Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

Preparation of extracts from tissue homogenates Tissue lysates are typically prepared by homogenization of tissue that is first minced and thoroughly rinsed in PBS to remove blood (dounce homogenizer recommended). Homogenize 100 to 200 mg of wet tissue in 500 µL – 1 mL of chilled 1X Cell Extraction Buffer PTR. For lower amounts of tissue adjust volumes accordingly. Incubate on ice for 20 minutes. Centrifuge at 18,000 x g for 20 minutes at 4ºC. Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and store at -80ºC. The sample protein concentration in the extract may be quantified using a protein assay. Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

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 30 minutes at room temperature on a plate shaker set to 750 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 30 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 50 µL of prepared Lumi HRP Development Solution to each well and incubate for 1 minute in the dark on a plate shaker set to 750 rpm. Further optimization of incubation time vs signal strength can be performed if needed. Avoid introducing bubbles into the wells.
8. Measure the produced light of each well using a microplate luminometer with the following settings: 0.5-1 second/well read time in summation mode (all wavelengths). Relative light unit (RLU) readings may vary between luminometer models. It is recommended to configure instrument settings according to the manufacturer's specifications. Note: Relative light unit (RLU) values may change over the course of the 15-minute reading window.
9. Analyze the data as described below.

Mode:	Luminescence
Instrument settings:	Endpoint
Detection Mode:	All wavelengths
Read Time:	0.5-1 sec
Read:	Top

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:
https://www.abcam.com/en-us/technical-resources/guides/elisa-guide

Technical Support
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Additional information

ASSAY SPECIFICITY

This kit is designed for the quantification of human IGFBP1.

The standard protein in this kit is full length human IGFBP1.

Native signal was detected in serum, plasma (citrate), plasma (EDTA), plasma (heparin), cell culture supernatant, urine, milk, cell extract, and tissue extract sample types.

Tissue supernatant samples have not been tested with this kit.

INTERFERENCE

50 ng/mL and 1.1 ng/mL of recombinant IGF1 and recombinant IGF2 were tested for interference. No interference was observed.

SPECIES REACTIVITY

Other species reactivity was determined by measuring 2% serum samples of various species, interpolating the protein concentrations from the human IGFBP1 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

- Preconfigured protocols are available when using SoftMax Pro software from Molecular Devices.
- Calculate the average chemiluminescence value for the blank control (zero) standards. Subtract the average blank control standard chemiluminescence value from all other chemiluminescence values.
- Create a standard curve by plotting the average blank control subtracted chemiluminescence 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 chemiluminescence 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 chemiluminescence 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 chemiluminescence values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at chemiluminescence 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)	RLU		Mean RLU
	1	2	
0	48	48	48
1.83	119	109	114
5.49	411	425	418
16.46	2,471	2,563	2,517
49.38	10,582	10,832	10,707
148.2	37,404	36,632	37,018
444.4	117,368	114,890	116,129
1,333	343,867	339,825	341,846
4,000	982,096	953,329	967,713

Table 1. Example of human IGFBP1 standard curve in Sample Diluent NS. The IGFBP1 standard curve was prepared as described in the Standard Preparation section. The table shows raw data values.

Standard Curve Measurements			
Concentration (pg/mL)	RLU		Mean RLU
	1	2	
0	41	41	41
1.83	112	114	113
5.49	331	335	333
16.46	2,351	2,241	2,296
49.38	11,406	11,381	11,394
148.2	39,263	39,067	39,165
444.4	123,647	125,644	124,646
1,333	355,503	355,469	355,486
4,000	984,143	983,181	983,662

Table 2. Example of human IGFBP1 standard curve in 1X Cell Extraction Buffer PTR. The IGFBP1 standard curve was prepared as described in the Standard Preparation section. The table shows raw data values.

TYPICAL SAMPLE VALUES

Sensitivity:

The minimal detectable dose (MDD) was determined by calculating the mean of zero standard replicates and adding 2 standard deviations then extrapolating the corresponding concentration.

Sample Diluent Buffer	n=	Minimal Detectable Dose
Sample Diluent NS	16	0.54 pg/mL
1X Cell Extraction Buffer PTR	16	0.45 pg/mL

Recovery

Three concentrations of IGFBP1 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 (%)
Serum [1:160]	99	94 - 104
Plasma – Citrate [1:160]	104	101 - 108
Plasma – EDTA [1:320]	103	97 - 111
Plasma – Heparin [1:160]	102	97 - 108
Urine [12.5%]	97	91 - 109
Milk [1.25%]	101	90 - 119
A549 Supernatant [50%]	98	81 - 107
HepG2 Supernatant [2.5%]	96	83 - 105
HepG2 Cell Extract [1.5 µg/ml]	100	89 - 112
U-87 MG Cell Extract [100 µg/ml]	98	83 - 115
Liver Tissue Homogenate Extract [200 µg/ml]	101	93 - 108

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 IGFBP1 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	2.5% Human Serum	5% Human Plasma (Citrate)	2.5% Human Plasma (EDTA)	5% Human Plasma (Heparin)
Undiluted	pg/mL	563	858	936	1,043
	% Expected value	100	100	100	100
2	pg/mL	287	421	477	521
	% Expected value	102	98	102	100
4	pg/mL	146	209	235	265
	% Expected value	104	98	101	102
8	pg/mL	69	105	115	136
	% Expected value	99	97	98	104
16	pg/mL	32	51	56	64
	% Expected value	92	95	95	98

Native IGFBP1 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	5% Human Milk	100% A549 Cell Supernatant	0.5% HepG2 Cell Supernatant
Undiluted	pg/mL	529	540	877
	% Expected value	100	100	100
2	pg/mL	272	251	441
	% Expected value	103	93	100
4	pg/mL	148	123	213
	% Expected value	112	91	97
8	pg/mL	77	56	104
	% Expected value	117	84	95
16	pg/mL	38	28	52
	% Expected value	114	82	94

Native IGFBP1 was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in 1X Cell Extraction Buffer PTR.

Dilution Factor	Interpolated value	4 µg/mL HepG2 Extract	500 µg/mL U-87 MG Extract	500 µg/mL Human Liver Homogenate Extract
Undiluted	pg/mL	643	430	828
	% Expected value	100	100	100
2	pg/mL	306	219	382
	% Expected value	95	102	92
4	pg/mL	145	114	184
	% Expected value	90	106	89
8	pg/mL	67	58	85
	% Expected value	83	107	82
16	pg/mL	33	31	43
	% Expected value	82	117	84

Recombinant IGFBP1 was spiked into the following biological samples and then diluted in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS.

Dilution Factor	Interpolated value	12.5% Human Urine
Undiluted	pg/mL	543
	% Expected value	100
2	pg/mL	283
	% Expected value	104
4	pg/mL	143
	% Expected value	105
8	pg/mL	69
	% Expected value	101
16	pg/mL	36
	% Expected value	107

Precision

Mean coefficient of variations of interpolated values of IGFBP1 from three concentrations of HepG2 Cell Supernatant within the working range of the assay.

	Intra-assay	Inter-assay
n=	5	3
CV (%)	5.1	6.3

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
https://www.abcam.com/en-us/technical-resources/guides/elisa-guide

Technical Support

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