

ab327288 – Human FGF1 SimpleStep ELISA® Kit, Chemiluminescent

For the quantitative measurement of FGF1 in human serum, cell culture supernatant, saliva, 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/ab327288

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 (ab219636).

Materials Supplied

Item	Quantity 1 x 96 tests	Quantity 10 x 96 tests	Storage Condition
Human FGF1 Capture Antibody 10X	600 µL	10 x 600 µL	+4°C
Human FGF1 Detector Antibody 10X	600 µL	10 x 600 µL	+4°C
Human FGF1 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	12 mL	2 x 50 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	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 to 1X with deionized water. To make 10 mL 1X Cell Extraction Buffer PTR combine 8 mL deionized water and 2 mL Cell Extraction Buffer PTR 5X. 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 **FGF1** protein standard by adding the volume indicated on the protein vial label. For **serum, saliva, and cell culture supernatant samples measurements**, use Sample Diluent NS. For **cell extract and tissue extract samples measurements**, use 1X Cell Extraction Buffer PTR. Hold at room temperature for 10 minutes and mix thoroughly and gently. This is the 2,500 pg/mL **Stock Standard #1** Solution.
- 2. Label eight tubes, Standards 1– 8.
- 3. Use the same Sample Diluent as used to resuspend the Stock Standard to prepare the standard curve. Add 150 µL of Sample Diluent into numbers 2-8.
- 4. Use the **Stock Standard #1** 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 #1	225	0	2,500	2,500
2	Stock Standard #1	75	150	2,500	833
3	Standard#2	75	150	833	278
4	Standard#3	75	150	278	92.6
5	Standard#4	75	150	92.6	30.9
6	Standard#5	75	150	30.9	10.3
7	Standard#6	75	150	10.3	3.43
8	Blank Control	0	150	0	0

Sample Preparation

Typical Sample Dynamic Range	
Sample Type	Range
Human Serum	≤ 50%
Human Saliva	≤ 50%
RPMI Culture Media	≤ 100%
PBMC Culture Supernatant	≤ 100%
Human Brain Extract	2.5 – 40 µg/mL
Mouse Brain Extract	2.5 – 20 µg/mL
Rat Brain Extract	5 – 20 µg/mL

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 1:2 into Sample Diluent NS and assay. Store un-diluted serum at -20°C 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. Assay, or dilute samples into Sample Diluent NS and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

Saliva Centrifuge saliva at 800 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.

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 4°C. Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and store at -80°C. 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 FGF1.

The standard protein in this kit is full-length mature human FGF1.

Native signal was detected in cell culture supernatant and tissue extract sample types.

Spiked protein experiments were used to validate serum, cell culture supernatant, and saliva sample types.

Milk and CSF samples have not been tested with this kit.

This kit is incompatible with plasma (citrate), plasma (EDTA), plasma (heparin), and urine samples.

CROSS REACTIVITY

Recombinant human FGFR1 was prepared at 5,000 pg/mL and assayed for cross reactivity. No cross-reactivity was observed.

INTERFERENCE

Recombinant human FGFR1 was prepared at 5,000 pg/mL and tested for interference with 500 pg/mL of FGF1. No interference with was observed.

SPECIES REACTIVITY

Recombinant mouse FGF1 was prepared at 1,000 pg/mL and 500 pg/mL and assayed for cross reactivity. An average of 79% cross-reactivity was observed.

The antibodies in this kit were raised against human FGF1 and additionally detect native signal in mouse and rat brain extract.

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	548	548	548
3.43	1,006	1,047	1,027
10.3	2,291	2,125	2,208
30.9	7,460	8,213	7,837
92.6	25,290	27,300	26,295
278	72,377	66,023	69,200
833	194,970	177,860	186,415
2,500	517,480	462,220	489,850

Table 1. Example of human FGF1 standard curve in Sample Diluent NS. The FGF1 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	548	548	548
3.43	1,072	1,001	1,037
10.3	2,291	2,125	2,208
30.9	7,460	8,213	7,837
92.6	25,290	27,300	26,295
278	72,377	66,023	69,200
833	194,970	177,860	186,415
2,500	517,480	462,220	489,850

Table 2. Example of human FGF1 standard curve in 1X Cell Extraction Buffer PTR. The FGF1 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	1.63 pg/mL
1X Cell Extraction Buffer PTR	16	2.13 pg/mL

Recovery

Three concentrations of FGF1 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 (%)
Human Serum (50%)	91	89 – 94
Human Saliva (50%)	88	87 – 91
RPMI Culture Media (100%)	114	112 – 115
Stimulated PBMC supernatant (100%)	103	101 – 105
Human Brain Extract (10 µg/mL)	103	101 – 104
Mouse Brain Extract (10 µg/mL)	104	103 – 105
Rat Brain Extract (10 µg/mL)	102	98 – 104

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 FGF1 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	40 µg/mL Human Brain Extract	20 µg/mL Mouse Brain Extract	20 µg/mL Rat Brain Extract
Undiluted	pg/mL	784	161	61
	% Expected value	100	100	100
2	pg/mL	445	90	34
	% Expected value	114	111	111
4	pg/mL	217	46	19
	% Expected value	111	113	123
8	pg/mL	117	24	ND
	% Expected value	120	119	ND
16	pg/mL	59	ND	ND
	% Expected value	121	ND	ND

ND – Not Detected – below product dynamic range

Recombinant FGF1 was spiked 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 Serum	50% Human Saliva	100% Stimulated PBMC Supernatant	100% RPMI Culture Media
Undiluted	pg/mL	401	429	547	547
	% Expected value	100	100	100	100
2	pg/mL	204	217	252	252
	% Expected value	102	101	92	92
4	pg/mL	109	112	123	123
	% Expected value	109	104	90	90
8	pg/mL	56	59	64	64
	% Expected value	112	110	94	94
16	pg/mL	30	30	33	33
	% Expected value	118	111	97	97

Precision

Mean coefficient of variations of interpolated values of FGF1 from three concentrations of human brain extract within the working range of the assay.

	Intra-assay	Inter-assay
N=	8	3
CV (%)	3.4	3.8

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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