Quantikine® ELISA

Human FGF basic/FGF2/bFGF Immunoassay

Catalog Number DFB50
SFB50
PDFB50

For the quantitative determination of human Fibroblast Growth Factor basic (FGF basic) concentrations in cell culture supernates, serum, and plasma.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

FGF basic, also called FGF2 (fibroblast growth factor-2) or HBGF-2 (heparin-binding growth factor-2), is the most intensively studied of the 22 mitogenic proteins of the FGF family (1-7). Family members share 35-60% amino acid (aa) identity, but only FGF acidic and basic lack signal peptides and are secreted by an alternate pathway. The 18 kDa FGF basic isoform can be found in both the cytoplasm and the nucleus and is also the form that is secreted (8-10). Storage pools within the cell or on cell surface heparan sulfate proteoglycans (HSPG) are likely (2). Transcription from alternate start sites produces 21-23 kDa forms found only in the nucleus (8, 9). High and low molecular weight human FGF basic isoforms target the expression of different genes (9, 10). The 18 kDa human FGF basic sequence shares 97% and 99% aa identity with mouse/rat and bovine/ovine FGF basic, respectively (6, 7). FGF basic is ubiquitously expressed. However, disruption of the mouse FGF basic gene gives relatively mild cardiovascular, skeletal, and neuronal phenotypes, suggesting compensation by other FGF family members (11-15). Transgenic over-expression of FGF basic mainly influences development and mineralization of bone (4, 16, 17).

Four FGF tyrosine kinase receptors (FGF R) and their splice variants show differential binding of FGFs (1). FGF basic preferentially binds FGF R1c and 2c, for which it has picomolar affinity (1, 2). FGF basic also has a number of other binding partners that fine-tune FGF basic activities, according to their locations and quantities. These include heparin, integrin αvβ3, soluble FGF R1, FGF-binding protein, free gangliosides, thrombospondin, pentraxin 3, fibrinogen, α2-macroglobulin, platelet-derived growth factor, and platelet factor-4, all of which bind with nanomolar affinity (2). These molecules may act as coreceptors or adhesion partners on cells, decoys or reservoirs in the extracellular matrix, and scavengers or chaperones as free proteins (2). Binding of FGF basic to cell surface HSPG is particularly critical, and is required for binding, dimerization and activation of FGF R (1, 2). FGF basic modulates such normal processes as angiogenesis, wound healing, tissue repair, learning and memory, and embryonic development and differentiation of heart, bone and brain (2-4). It is upregulated in response to inflammation via mediators such as TNF-α, IL-1β, IL-2, PDGF, and nitric oxide (2). Many human tumors express FGF basic, which may correlate with tumor vascularity (2, 5).

The Quantikine® Human FGF basic/FGF2/bFGF Immunoassay kit is a 4.5 hour solid phase ELISA designed to measure human FGF basic in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant human FGF basic and antibodies raised against the recombinant factor. It has been shown to quantitate recombinant human FGF basic accurately. Results obtained using natural human FGF basic showed linear curves that were parallel to the standard curves obtained using the Quantikine® kit standards. These results indicate that this kit can be used to determine relative mass values for natural human FGF basic.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human FGF basic has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any FGF basic present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for human FGF basic is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of FGF basic bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, dilute the samples with the calibrator diluent and repeat the assay.
- Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine® Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	CATALOG # DFB50	CATALOG # SFB50	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human FGF basic Microplate	890656	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human FGF basic.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
Human FGF basic Standard	890658	2 vials	12 vials	Recombinant human FGF basic in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Use a fresh standard for each assay. Discard the FGF basic stock solution and dilutions after 4 hours.
Human FGF basic Conjugate	890657	1 vial	6 vials	21 mL/vial of a monoclonal antibody specific for human FGF basic conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-43	895521	1 vial	6 vials	11 mL/vial of a buffered protein base with preservatives and blue dye. *Contains a precipitate. Mix well before and during use.*	
Calibrator Diluent RD5-14	895300	1 vial	6 vials	21 mL/vial of a buffered protein base with preservatives.	
Wash Buffer Concentrate	895003	1 vial	6 vials	21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	1 vial	6 vials	12 mL/vial of stabilized hydrogen peroxide.	
Color Reagent B	895001	1 vial	6 vials	12 mL/vial of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	1 vial	6 vials	6 mL/vial of 2 N sulfuric acid.	
Plate Sealers	N/A	4 strips	24 strips	Adhesive strips.	

* Provided this is within the expiration date of the kit.

DFB50 contains sufficient materials to run an ELISA on one 96 well plate.
SFB50 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PDFB50). Refer to the PharmPak Contents section for specific vial counts.

PHARMPAK CONTENTS

Each PharmPak contains reagents sufficient for the assay of 50 microplates (96 wells/plate). The package inserts supplied are the same as those supplied in the single kit packs and because of this, a few minor differences related to the number of reagents and their container sizes should be noted.

- Sufficient material is supplied to perform at least 50 standard curves; reuse of each vial may be required. The number of vials, and the number of standard curves obtained per vial will vary with the analyte.
- Wash Buffer 25X Concentrate is bulk packed in 125 mL bottles containing 100 mL.
  Note: *Additional wash buffer is available for purchase (R&D Systems®, Catalog # WA126).*

The reagents provided in this PharmPak are detailed below.

PART	PART #	QUANTITY
Human FGF basic Microplate	890656	50 plates
Human FGF basic Standard	890658	50 vials
Human FGF basic Conjugate	890657	50 vials
Assay Diluent RD1-43	895521	50 vials
Calibrator Diluent RD5-14	895300	50 vials
Wash Buffer Concentrate	895126	9 bottles
Color Reagent A	895000	50 vials
Color Reagent B	895001	50 vials
Stop Solution	895032	50 vials
Plate Sealers	N/A	100 sheets
Package Insert	750336	2 booklets

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm
- Pipettes and pipette tips
- 500 mL graduated cylinder
- Deionized or distilled water
- Squirt bottle, manifold dispenser, or automated microplate washer
- Test tubes for dilution of standards
- Human FGF basic Controls (optional; R&D Systems®, Catalog # QC20)

PRECAUTIONS

Calibrator Diluent RD5-14 and the Human FGF basic Standard contain sodium azide which may react with lead and copper plumbing to form explosive metallic azides. Flush with large quantities of water during disposal.

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the SDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -70 °C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -70 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -70 °C. Avoid repeated freeze-thaw cycles.

Note: *Heparin and citrate plasma are not recommended for use in this assay.*
Grossly hemolyzed or icteric samples are not suitable for use in this assay.

REAGENT PREPARATION

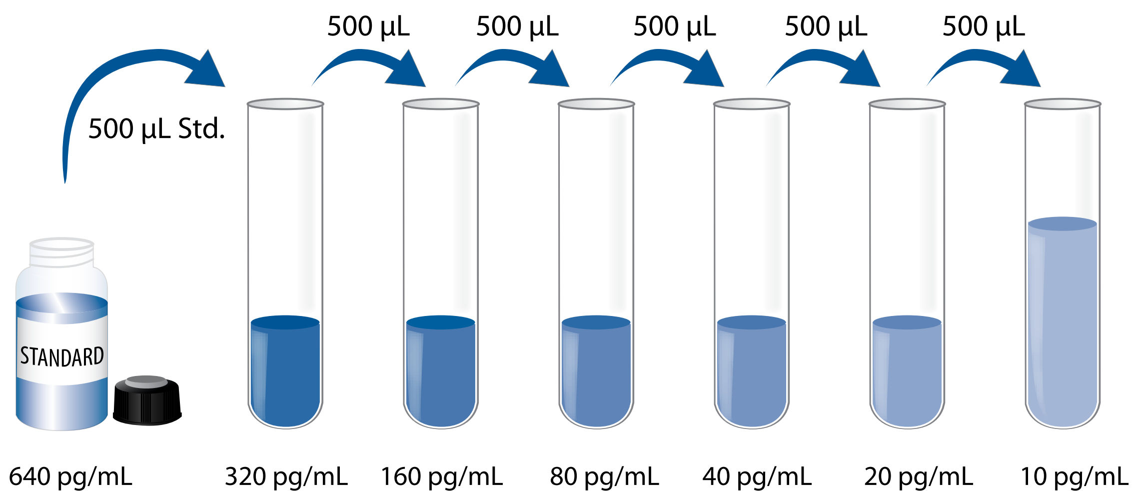
Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 μL of the resultant mixture is required per well.

Human FGF basic Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human FGF basic Standard with Calibrator Diluent RD5-14. This reconstitution produces a stock solution of 640 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 500 μL of Calibrator Diluent RD5-14 into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Human FGF basic Standard (640 pg/mL) serves as the high standard. Calibrator Diluent RD5-14 serves as the zero standard (0 pg/mL). Discard the FGF basic stock solution and dilutions after 4 hours. Use a fresh standard for each assay.



For research use only. Not for use in diagnostic procedures.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, controls, and samples be assayed in duplicate.

1. Prepare all reagents and working standards 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, and reseal.
3. Add 100 µL of Assay Diluent RD1-43 to each well. *Assay Diluent RD1-43 contains a precipitate. Mix well before and during its use.*
4. Add 100 µL of standard, control, or sample per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature. A plate layout is provided to record samples and standards assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 200 µL of Human FGF basic Conjugate to each well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**
9. Add 50 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

CALCULATION OF RESULTS

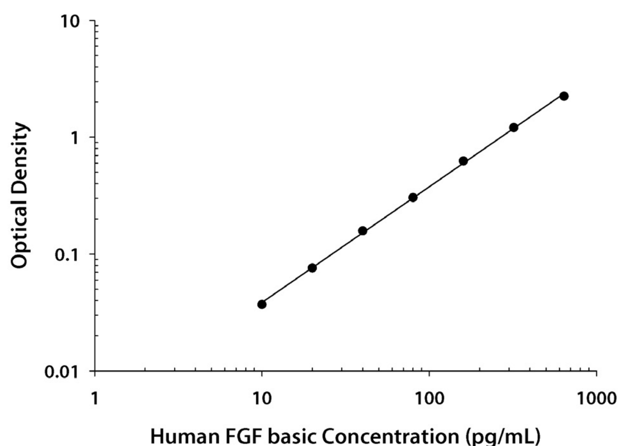
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a log/log curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on a log/log graph. The data may be linearized by plotting the log of the human FGF basic concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D.	Average	Corrected
0	0.063 0.061	0.062	—
10	0.100 0.098	0.099	0.037
20	0.139 0.136	0.138	0.076
40	0.223 0.216	0.220	0.158
80	0.367 0.366	0.366	0.304
160	0.688 0.686	0.687	0.625
320	1.274 1.264	1.269	1.207
640	2.310 2.291	2.300	2.238

PRECISION

Intra-Assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-Assay Precision (Precision between assays)
Three samples of known concentration were tested in forty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (pg/mL)	28.8	104	234	32.8	108	229
Standard deviation	2.8	3.2	7.1	3.0	8.0	17.3
CV (%)	9.7	3.1	3.0	9.1	7.4	7.6

RECOVERY

The recovery of human FGF basic spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	108	94-119%
Serum (n=5)	106	86-124%
EDTA plasma (n=5)	103	90-124%

LINEARITY

To assess the linearity of the assay, samples were spiked with high concentrations of human FGF basic and diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=4)	Serum (n=5)	EDTA plasma (n=5)
1:2	Average % of Expected	102	92	89
	Range (%)	99-103	81-114	81-109
1:4	Average % of Expected	100	97	92
	Range (%)	98-104	88-115	82-108
1:8	Average % of Expected	102	98	93
	Range (%)	92-122	88-114	82-108
1:16	Average % of Expected	93	90	91
	Range (%)	87-99	81-106	80-111

SENSITIVITY

The minimum detectable dose (MDD) of human FGF basic is typically less than 3 pg/mL.

The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against a highly purified *E. coli*-expressed recombinant human FGF basic produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of human FGF basic in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Serum	ND	0	ND
EDTA plasma	13.5	10	ND-14.6

ND=Non-detectable

Cell Culture Supernates:

Human peripheral blood mononuclear cells (5 x 10^6 cells/mL) were cultured in RPMI supplemented with 5% fetal bovine serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. The cells were stimulated with 10 µg/mL PHA. Aliquots of the culture supernates were removed on days 1 and 5 and assayed for levels of natural human FGF basic. All samples measured below the lowest standard, 10 pg/mL.

K562 human chronic myelogenous leukemia cells (4 x 10^4 cells/mL) were cultured in RPMI supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. An aliquot of the cell culture supernate was removed on day 3, assayed for natural human FGF basic, and measured 34 pg/mL.

PC-3 human prostate cancer cells were cultured in MEM supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. The cells were stimulated with 10 µg TNF-α. An aliquot of the cell culture supernate was removed, assayed for natural human FGF basic, and measured 27 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant human (rh) FGF basic.

The factors listed below were prepared at 50 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range rhFGF basic control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

β-ECGF
EGF
FGF-3
FGF-4
FGF-5
FGF-6
FGF-8a
FGF-8e
FGF-8f
FGF-9
FGF-10
FGF-12
FGF-16
FGF-17
FGF-18
FGF-19
FGF-20
FGF-21
FGF-22
FGF-23
FGF acidic
FGF-BP
FGF R1α (IIIb)
FGF R1α (IIIc)
FGF R1β (IIIb)
FGF R1β (IIIc)
FGF R2α (IIIb)
FGF R2α (IIIc)
FGF R2β (IIIb)
FGF R2β (IIIb)
FGF R2β (IIIc)
FGF R3 (IIIb)
FGF R3 (IIIc)
FGF R4
G-CSF
GM-CSF
HB-EGF
HGF
IGF-I
IGF-II
KGF/FGF-7
Klotho
Klotho β
M-CSF
β-NGF
PD-ECGF
PDGF-AA
PDGF-AB
PDGF-BB
Pentraxin 3/TSG-14
VEGF

Recombinant mouse:

FGF-4
FGF-6
FGF-8b
FGF-8c
FGF-9
FGF-10
FGF-23
FGF acidic
FGF R2β (IIIb)
FGF R2β (IIIc)
FGF R3 (IIIc)
GM-CSF
KGF/FGF-7
Klotho
Klotho β
Pentraxin 3/TSG-14

Recombinant rat:

FGF-BP

Natural proteins:

human PDGF
porcine PDGF

Cross-reactivity was observed with the following:

Factor	% Cross-reactivity
Natural bovine FGF basic	40
Natural bovine FGF acidic	0.15
Recombinant bovine FGF basic	17
Recombinant mouse FGF basic	18
Recombinant rat FGF basic	57

Other FGF binding proteoglycans - Soluble sodium heparin (from bovine intestinal mucosa) at concentrations of 100 ng/mL, 1 µg/mL, and 100 µg/mL was assayed for cross-reactivity and interference. No cross-reactivity or interference was observed.

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PLATE LAYOUT

Use this plate layout to record standards and samples assayed.

	A	B	C	D	E	F	G	H
12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								

NOTES

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