FGF2 (Human) ELISA Kit

Catalog Number KA0527

96 assays

Version: 04

Intended for research use only

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Table of Contents

Introduction

Intended Use

This ELISA Kit is to be used for the in vitro quantitative determination of human basic fibroblast growth factor (bFGF) concentrations in serum, plasma, cell culture supernatant, urine, and other biological fluids. This kit is intended FOR LABORATORY RESEARCH USE ONLY and is not to be used for diagnostic or therapeutic procedures.

Background

When proteins synthesized by one cell can diffuse over small distances to induce changes in neighboring cells, the event is called a **paracrine interaction**, and the diffusible proteins are called **paracrine factors** or **growth and differentiation factors** (**GDFs**). Many of these paracrine factors can be grouped into four major families on the basis of their structures. These families are the **fibroblast growth factor (FGF)** family, the Hedgehog family, the Wingless (Wnt) family, and the TGF-β superfamily.

The **fibroblast growth factor** (**FGF**) family currently has over a dozen structurally related members. FGF-1 is also known as acidic FGF; FGF-2 is called basic FGF (bFGF); and FGF7 sometimes goes by the name of keratinocyte growth factor. Over a dozen distinct FGF genes are known in vertebrates, and they can generate hundreds of protein isoforms by varying their RNA splicing or initiation codons in different tissues. FGFs can activate a set of receptor tyrosine kinases called the **fibroblast growth factor receptors** (**FGFRs**). The receptor tyrosine kinases are proteins that extend through the cell membrane. On the extracellular side is the portion of the protein that binds the paracrine factor. On the intracellular side is a dormant tyrosine kinase (i.e., a protein that can phosphorylate another protein by splitting ATP). When the FGF receptor binds an FGF (and only when it binds an FGF), the dormant kinase is activated, and it phosphorylates certain proteins within the responding cell. The proteins are now activated and can perform new functions. FGFs are associated with several developmental functions, including angiogenesis (blood vessel formation), mesoderm formation, and axon extension. While FGFs can often substitute for one another, their expression patterns give them separate functions. Basic FGF especially important in angiogenesis, and FGF8 is important for the development of the midbrain and limbs.

The FGFs have bee showed 30-505 overall sequence homology at the amino acid level. They are present at significantly higher concentrations than the neurotrophins; FGF-1 and bFGF concentrations, respectively, are approximately 500-fold and 50-fold greater than that of NGF. The major FGF translation products do not possess a signal peptide sequence and are found principally within the cytoplasm of cells in which they are expressed. Thus, like several other growth factors, it is not entirely. It is suspected that other members of the FGF family influence development. For example, there is evidence that FGF-8 is involved in axial specification and patterning of limb development. FGFs stimulate the proliferation of neurons in the developing nervous

system and glial cells throughout life. The bFGF stimulates the proliferation of multipotential stem cells, which subsequently give rise to neurons of the cortex .The FGFs also exhibit trophic activity toward mature neurons, promoting the survival of these cells without stimulating DNA synthesis. There is some evidence that the FGFs may play a critical role in facilitating axonal regeneration in the PNS and provide trophic support to neurons following trauma or injury.

The complexity of FGF action is compounded by the existence of at least four receptors for FGF (FGFR1–4). Three FGFRs are expressed in the CNS, where they exist as multiple alternatively spliced products. All of the FGFRs are ligand-activated tyrosine kinases and comprise a distinct subfamily of the receptor tyrosine kinases. The interaction of the various FGFs with the four FGFRs and their multiple mRNA splice products is bewilderingly complex and incompletely understood. FGFR1 appears to be expressed exclusively in neurons, while principally glial cells express bFGFR and FGFR3. Interestingly, neurons of the substantia nigra and some motor neurons appear to express both FGF-1 and its receptor, FGFR1, suggesting that FGF-1 may act in an autocrine fashion to support these cells. Both receptors are expressed prior to the appearance of their ligands, FGF-1 and bFGF. These data support the view that other FGF family members are more functionally relevant species during embryogenesis. FGFR1 is expressed in the primitive neuroepithelium. A novel aspect of FGF biology is the ability of these growth factors to bind to cell surface proteoglycans, specifically heparan sulfate proteoglycans. Indeed, it appears that these proteoglycans can act as low-affinity receptors for the FGFs. It is thought that FGFs bind to the proteoglycans, which effectively immobilize them and induce or stabilize an active conformation, facilitating binding to the FGFR.

bFGF is the most extensively studied member in FGF family, and it found in almost all tissues of mesodermal and neuroectodermal origin and also in tumors related to these tissues. Endothelial cells produce large amounts of this factor. Some bFGF is associated with the extracellular matrix of the subendothelial cells. bFGF is an 18 kDa protein with a length of 155 amino acids and an isoelectric point of 9.6. It does not contain disulfide bonds and is not glycosylated. bFGF stimulates the growth of fibroblasts, myoblasts, osteoblasts, neuronal cells, endothelial cells, keratinocytes, chondrocytes, and many other cell types. bFGF has been shown to be a promoting or inhibitory modulator of cellular differentiation also for other cell types. bFGF is not only a mitogen for chondrocytes but also inhibits their terminal differentiation. It was proved the relationship between the bFGF expression and the advancement level of colorectal cancer and the survival of the patients' ill with this disease. It was stated that the highest levels of bFGF was connected with the advancement of neoplastic process, especially when metastases coexisted at+ the same time. It was also demonstrated that the higher levels of bFGF gave worse prognosis as far as survival was concerned. These observations suggest the bFGF and it's family may play an active role in variety of pathological condition.

This human bFGF test kit is a quantitative measurement of human bFGF in serum and cell culture supernatants using ELISA (Enzyme-Linked Immunosorbent Assay) in vitro.

Principle of the Assay

This bFGF enzyme-linked immunosorbent assay (ELISA) applies a technique called a quantitative sandwich immunoassay. The microtiter plate provided in this kit has been pre-coated with a monoclonal specific for bFGF. Standards or samples are then added to the appropriate microtiter plate wells and incubated. bFGF if present, will bind and become immobilized by the antibody pre-coated on the wells. The microtiter plate wells are thoroughly washed to remove unbound bFGF and other components of sample. In order to quantitative the amount of bFGF present in the sample, a standardized preparation of horseradish peroxidase (HRP)-conjugated polyclonal antibody specific for bFGF is added to each well to "sandwich" the bFGF immobilised during the first incubation. The microtiter plate then undergoes a second incubation. The wells are thoroughly washed to remove all unbound HRP-conjugated antibodies and a TMB (3,3'5,5' tetramethyl-benzidine) substrate solution is added to each well. The enzyme (HRP) and substrate are allowed to react over a short incubation period. Only those wells that contain bFGF and enzyme-conjugated antibody will exhibit a change in colour. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the colour change is measured by spectrophotometer at a wavelength of 450 nm ± 2 nm.

In order to measure the concentration of bFGF in the samples, this kit contains two calibration diluents (Calibrator Diluent I for serum/plasma testing and Calibrator Diluent II for cell culture supernatant/ urine testing). According to the testing system, the provided standard is diluted (2-fold) with the appropriate Calibrator Diluent and assayed at the same time as the samples. This allows the operator to produce a standard curve of Optical Density (O.D.) versus bFGF concentration (pg/ml). The concentration of bFGF in the samples is then determined by comparing the O.D. of the samples to the standard curve.

General Information

Materials Supplied

List of component

Component	State	Amount
bFGF Microtiter plate	Pre-coated with anti-human bFGF monoclonal antibody	96 wells
Biotin Conjugate	Anti-human bFGF monoclonal antibody conjugated to Biotin	7 mL
Avidin Conjugate	Avidin conjugated to horseradish peroxidase	14 mL
bFGF Standard	Recombinant human bFGF (16 ng/vial) in a buffered protein base with preservative, lyophilized.	2 vials
Calibrator diluent I	Animal serum with preservative. For serum/plasma testing	25 mL
Calibrator diluent II	Cell culture medium with calf serum and preservative. For cell culture supernatant/urine testing.	25 mL
Wash Buffer (20x)	20-fold concentrated solution of buffered surfactant	60 mL
Substrate A	Buffered solution with H_2O_2	10 mL
Substrate B	Buffered solution with TMB.	10 mL
Stop solution	2N Sulphuric Acid (H_2SO_4). Caution: Caustic Material!	14 mL

Storage Instruction

All reagents provided are stored at 2-8℃. Refer to the expiration date on the label.

Materials Required but Not Supplied

1. Single or multi-channel precision pipettes with disposable tips: 10-100µl and 50-200µl for running the assay.
2. Pipettes: 1 ml, 5 ml 10 ml, and 25 ml for reagent preparation.
3. Multi-channel pipette reservoir or equivalent reagent container.
4. Test tubes and racks.
5. Polypropylene tubes or containers (25 ml).
6. Erlenmeyer flasks: 100 ml, 400 ml, 1 L and 2 L
7. Microtiter plate reader (450 nm ± 2nm)
8. Automatic microtiter plate washer or squirt bottle.
9. Sodium hypochlorite solution, 5.25% (household liquid bleach).
10. Deionized or distilled water.
11. Plastic plate cover.
12. Disposable gloves.
13. Absorbent paper.

Precautions for Use

- Precautions

✓ Do not substitute reagents from one kit lot to another. Standard, conjugate and microtiter plates are matched for optimal performance. Use only the reagents supplied by manufacturer.

✓ Allow kit reagents and materials to reach room temperature (20-25℃) before use. Do not use water baths to thaw samples or reagents.

✓ Do not use kit components beyond their expiration date.

✓ Use only deionized or distilled water to dilute reagents.

✓ Do not remove microtiter plate from the storage bag until needed. Unused strips should be stored at 2-8℃ in their pouch with the desiccant provided.

✓ Use fresh disposable pipette tips for each transfer to avoid contamination.

✓ Do not mix acid and sodium hypochlorite solutions.

✓ Human serum and plasma should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure, since no known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.

✓ All samples should be disposed of in a manner that will inactivate human viruses.
Solid Wastes: Autoclave for 60 minutes at 121℃.
Liquid Wastes: Add sodium hypochlorite to a final concentration of 1.0%. The waste should be allowed to stand for a minimum of 30 minutes to inactivate the virus before disposal.

✓ Substrate Solution is easily contaminated. If bluish prior to use, do not use.

✓ Substrate B contains 20% acetone, keep this reagent away from sources of heat or flame.

✓ If Wash Buffer (20X) is stored at a lower temperature (2-5℃), crystals may form, which must be dissolved by warming to 37℃ prior to use.7

Assay Protocol

Reagent Preparation

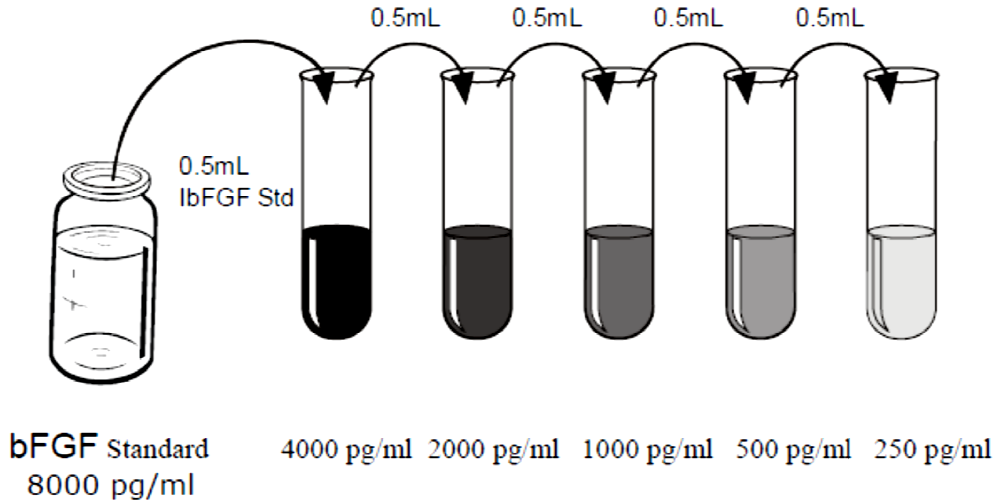
Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25℃). Prepare the following reagents as indicated below. Mix thoroughly by gently swirling before pipetting. Avoid foaming.

- Wash Buffer (1X): Add 60 ml of Wash Buffer (20X) and dilute to a final volume of 1200 mL with distilled or deionized water. Mix thoroughly. If a smaller volume of Wash Buffer (1X) is desired, add 1 volume of Wash Buffer (20X) to 19 volumes of distilled or deionized water. Wash Buffer (1X) is stable for 1 month at 2-8℃. Mix well before use.

- Substrate Solution: Substrate A and Substrate B should be mixed together in equal volumes up to 15 minutes before use. Refer to the table below for correct amounts of Substrate Solution to prepare.

Strips Used	Substrate A (mL)	Substrate B (mL)	Substrate Solution (mL)
2 strips (16 wells)	1.5	1.5	3.0
4 strips (32 wells)	3.0	3.0	6.0
6 strips (48 wells)	4.0	4.0	8.0
8 strips (64 wells)	5.0	5.0	10.0
10 strips (80 wells)	6.0	6.0	12.0
12 strips (96 wells)	7.0	7.0	14.0

- bFGF Standard: Two vials of Standards are provided in this kit to allow both serum/plasma and cell culture supernatant testing. Reconstitute the bFGF Standard with either 2.0 ml of Calibrator Diluent I (for serum/plasma testing) or Calibrator Diluent II (for cell culture supernatant/urine testing). This reconstitution produces a stock solution of 8000 pg/ml. Allow solution to sit for at least 15 minutes with gentle agitation prior to making dilutions. Use within one hour of reconstituting. The bFGF standard stock solution can be stored frozen (-20℃) for up to 30 days. Avoid freeze-thaw cycles; aliquot if repeated use is expected.
- Use the above stock solution to produce a serial 2-fold dilution series within the range of this assay (250 pg/ml to 8000 pg/ml) as illustrated. Add 0.5 ml of the appropriate Calibrator Diluent to each test tube. Between each test tube transfer be sure to mix contents thoroughly. The undiluted bFGF Standard will serve as the high standard (8000 pg/ml) and the Calibrator Diluent will serve as the zero standard (0 pg/ml).



Sample Preparation

- Cell Culture Supernatant: Centrifuge to remove any visible particulate material.
- Serum: Blood should be drawn using standard venipuncture techniques and serum separated from the blood cells as soon as possible. Samples should be allowed to clot for one hour at room temperature, centrifuged for 10 minutes (4℃) and serum extracted.
- Plasma: Blood should be drawn using standard venipuncture techniques and plasma collected using sodium citrate, EDTA, or heparin as an anticoagulant. To ensure optimal recovery and minimal platelet contamination, after collection there must be quick separation of plasma with less than 30 minutes on ice. Centrifuge for 10 minutes (4℃) to remove any particulate. This bFGF ELISA kit is not affected by haemolysis of specimens. No adverse effects have been noted in the presence of anti-coagulants, sodium citrate, EDTA, or heparin.

Note:

✓ *When performing the assay slowly bring samples to room temperature.*

✓ *It is recommended that all samples be assayed in duplicate.*

✓ *DO NOT USE HEAT-TREATED SPECIMENS.*

Assay Procedure

1. Prepare Wash Buffer and bFGF Standards before starting assay procedure (see Preparation of Reagents). It is recommended that the table and diagram provided be used as a reference for adding Standards or Samples to the Microtiter Plate.

Wells	Contents	Wells	Contents
1A, 1B	Standard 1 - **0 pg/ml** (S1)	**2A, 2B**	Standard 5 - **2000 pg/ml**(S5)
1C, 1D	Standard 2 - **250 pg/ml**(S2)	**2C, 2D**	Standard 6 - **4000 pg/ml**(S6)
1E, 1F	Standard 3 - **500 pg/ml**(S3)	**2E, 2F**	Standard 7 - **8000 pg/ml**(S7)
1G, 1H	Standard 4 - **1000 pg/ml**(S4)	**2G,12H**	bFGF samples

2. Add 100 µl of Standard or sample to the appropriate well of the antibody pre-coated Microtiter Plate and incubate 1 hour at room temperature.
3. Without discarding the standards and samples, add 50 µl bFGF Biotin conjugate to each wells. Mix well. Cover and incubate for 1 hour at room temperature.
4. Wash the Microtiter Plate using one of the specified methods indicated below:

   Manual Washing: Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Using a squirt bottle, fill each well completely with Wash Buffer (1X) then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure four more times for a **total of FIVE washes**. After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. Note: Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame.

   Automated Washing: Aspirate all wells, then wash plates **FIVE times** using Wash Buffer (1X). Always adjust your washer to aspirate as much liquid as possible and set fill volume at 350 µlwell/wash (range: 350-400 µl. After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. It is recommended that the washer be set for a soaking time of 10 seconds or shaking time of 5 seconds between washes.
5. Dispense 100µL of Avidin conjugate to each well Mix well. Cover and incubate for 1 hour at room temperature.
6. Prepare Substrate Solution no more than 15 minutes before end of second incubation (see Preparation of Reagents).
7. Repeat wash procedure as described in Step 4.
8. Add 100 µl Substrate Solution to each well. Cover and incubate for 15 minutes at room temperature.
9. Add 100 µl Stop Solution to each well. Mix well.
10. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader set within 30 minutes.

Data Analysis

Calculation of Results

The standard curve is used to determine the amount of bFGF in an unknown sample. The standard curve is generated by plotting the average O.D. (450 nm) obtained for each of the standard concentrations on the vertical (Y) axis versus the corresponding bFGF concentration (pg/ml) on the horizontal (X) axis.

1. First, calculate the mean O.D value for each standard and sample. All O.D. values are subtracted by the value of the zero-standard (0 pg/ml) before result interpretation. Construct the standard curve using graph paper or statistical software.
2. To determine the amount of bFGF in each sample, first locate the O.D. value on the Y-axis and extend a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the corresponding bFGF concentration.
3. If samples generate values higher than the highest standard, dilute the samples with the appropriate Calibrator Diluent and repeat the assay, the concentration read from the standard curve must be multiplied by the dilution factor.

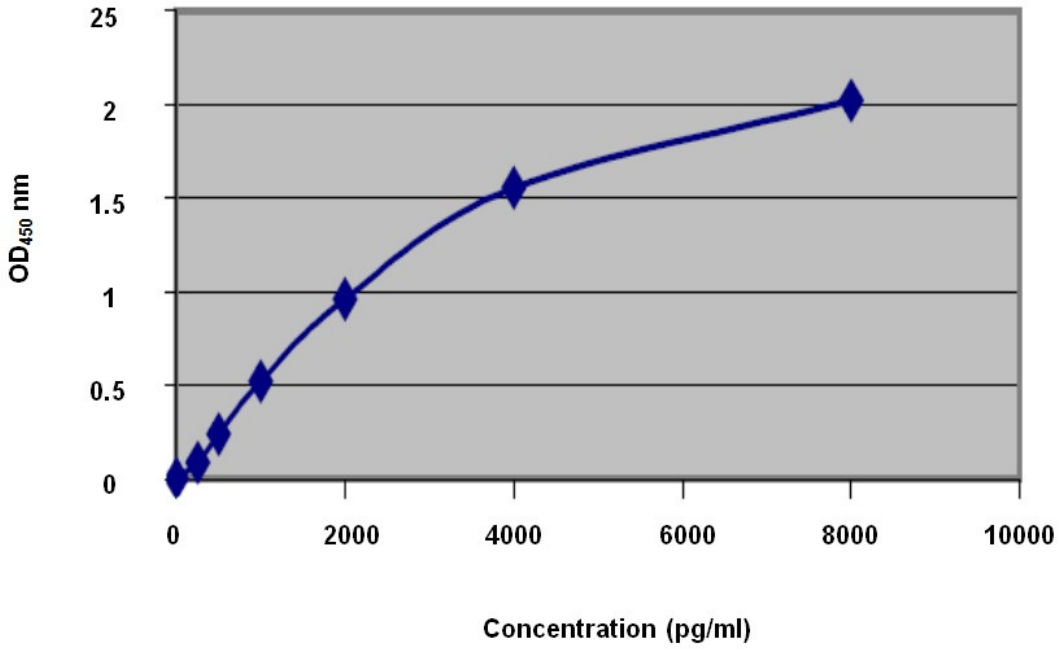
Typical data

Results of a typical standard run of a bFGF ELISA are shown below. Any variation in standard diluent, operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variation in result. The following examples are for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain their own standard curve

Example one

The following data was obtained for a standard curve using Calibrator Diluent II.

Standard (pg/ml)	O.D. (450 nm)	Mean	Zero Standard Subtracted (Std.) -(S1)
0	0.036, 0.037	0.0365	0
250	0.130, 0.131	0.1305	0.094
500	0.247, 0.225	0.2360	0.200
1000	0.518, 0.518	0.5180	0.481
2000	1.956, 0.966	0.9610	0.926
4000	1.521, 1.585	1.5530	1.516
8000	1.989, 2.052	2.021	1.985



Performance Characteristics

- Inter-assay precision

To determine within-run precision, three different samples of known concentration were assayed by using 10 replicates in 1 assay.

Calibrator Diluent assay			
Sample	1	2	3
n	10	10	10
Mean (pg/mL)	142	481	2044
Standard Deviation (pg/mL)	3.5	26.7	124.5
Coefficient of Variation (%)	2.44	5.55	6.09

- Intra-Assay Precision

To determine between-run precision, three different samples of known concentration were assayed by using replicates on different assays.

Calibrator Diluent assay			
Sample	1	2	3
n	20	20	20
Mean (pg/mL)	148.8	473.4	2035
Standard Deviation (pg/mL)	7.7	25.6	175.4
Coefficient of Variation (%)	5.16	5.4	8.62

- Recovery

The recovery of bFGF spiked to 3 different levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average Range %	Range %
Cell culture media	108	93-117
Serum	94	85-105
Plasma	102	96-108

- Sensitivity

The minimum detectable dose of bFGF was determined by adding two standard deviations to the mean optical density value of the 20 zero standard replicates and calculating the corresponding concentration from the standard curve. The minimum detectable dose using a standard curve generated with Calibrator Diluent I is 80 pg/ml and using Calibrator Diluent II is 78 pg/ml.

- Specificity

This sandwich ELISA recognises both natural and recombinant human bFGF. This kit exhibits no detectable cross-reactivity with human; TGF-ß, GM-CSF, MCP-3, INF-r, EGF, IL-1α, IL-8, IL-1ß, TNF-α, IL-16, MCSF, IL-5, and IL-16.

- Calibration

This immunoassay is calibrated against NIBSC Standard (90/712).

Resources

Reference

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Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1 0 pg/ml	S5 2000 pg/ml										
B	S1 0 pg/ml	S5 2000 pg/ml										
C	S2 250 pg/ml	S6 4000 pg/ml										
D	S2 250 pg/ml	S6 4000 pg/ml										
E	S3 500 pg/ml	S7 8000 pg/ml										
F	S3 500 pg/ml	S7 8000 pg/ml										
G	S4 1000 pg/ml											
H	S4 1000 pg/ml											