ab99980 – FGF basic (FGF2) Human ELISA Kit

Instructions for Use

For the quantitative measurement of Human FGF basic in cell lysates and tissue lysates.

This product is for research use only and is not intended for diagnostic use.

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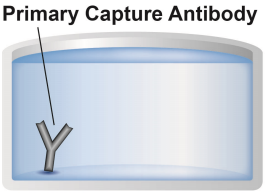
1. BACKGROUND

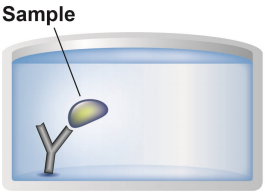
Abcam's FGF basic (FGF2) Human ELISA (Enzyme-Linked Immunosorbent Assay) kit is an *in vitro* enzyme-linked immunosorbent assay for the quantitative measurement of Human FGF basic in cell lysates and tissue lysates.

This assay employs an antibody specific for Human FGF basic coated on a 96-well plate. Standards and samples are pipetted into the wells and FGF basic present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-Human FGF basic antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated Streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of FGF basic bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

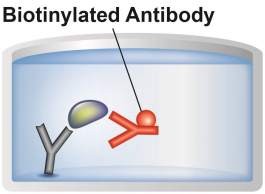
2. ASSAY SUMMARY



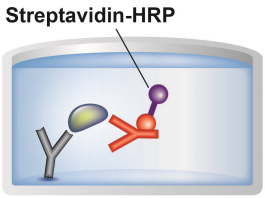
Prepare all reagents, samples and standards as instructed.



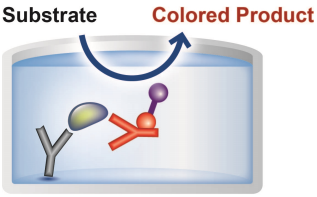
Add standard or sample to each well used. Incubate at room temperature



Add prepared biotin antibody to each well. Incubate at room temperature.



Add prepared Streptavidin solution. Incubate at room temperature.



Add TMB One-Step Development Solution to each well. Incubate at room temperature. Add Stop Solution to each well. Read at 450nm immediately.

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at -20°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 section 9. Reagent Preparation.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
FGF basic Microplate (12 x 8 wells)	96 wells	-20°C
20X Wash Buffer Concentrate	25 mL	-20°C
Recombinant Human FGF basic Standard	2 vials	-20°C
5X Sample Diluent Buffer	10 mL	-20°C
5X Assay Diluent	15 mL	-20°C
Biotinylated anti-Human FGF basic	2 vial	-20°C
120X HRP-Streptavidin Concentrate	200 µL	-20°C
TMB One-Step Substrate Reagent	12 mL	-20°C
Stop Solution	8 mL	-20°C
2X Cell Lysis Buffer	5mL	-20°C

6. 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 nm.
- Precision pipettes to deliver 2 µL to 1 mL volumes.
- Adjustable 1-25 mL pipettes for reagent preparation.
- 100 mL and 1 liter graduated cylinders.
- Absorbent paper.
- Distilled or deionized water.
- Log-log graph paper or computer and software for ELISA data analysis.
- Tubes to prepare standard or sample dilutions.

7. LIMITATIONS

- Do not mix or substitute reagents or materials from other kit lots or vendors.

8. TECHNICAL HINTS

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps.
- When preparing your standards, it is very critical to briefly spin down the vial first. The powder may drop off from the cap when opening it if you do not spin down. Be sure to dissolve the powder thoroughly when reconstituting. After adding Assay Diluent to the vial, we recommend inverting the tube a few times, then flick the tube a few times, and then spin it down; repeat this procedure 3-4 times. This is a technique we find very effective for thoroughly mixing the standard without too much mechanical force.
- Do not vortex the standard during reconstitution, as this will destabilize the protein.
- Once your standard has been reconstituted, it should be used right away or else frozen for later use.
- Keep the standard dilutions on ice while during preparation, but the ELISA procedure should be done at room temperature.
- Be sure to discard the working standard dilutions after use – they do not store well.
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**

9. REAGENT PREPARATION

Equilibrate all reagents to room temperature (18-25°C) prior to use.

9.1 **1X Sample Diluent Buffer**

5X Sample Diluent Buffer should be diluted 5-fold with deionized or distilled water before use.

9.2 **1X Assay Diluent**

5X Assay Diluent should be diluted 5-fold with deionized or distilled water before use.

9.3 **1X Cell Lysis**

2X Cell Lysis Buffer should be diluted 2-fold with deionized or distilled water (for cell lysate and tissue lysate).

*When it comes to the actual use of this reagent for sample preparation, the methods vary depending on sample type/appearance. The choices of the method for lysis and homogenization include glass-bead "smash," douncing, freeze/thaw, sonication and crushing frozen tissue with a mortar and pestle, or even a combination of these. There is no best method for all sample types; your choice of method should be made following a brief search of the literature to see how samples similar to yours have been prepared in previous investigations.

9.4 **1X Wash Solution**

If the 20X Wash Concentrate contains visible crystals, equilibrate to room temperature and mix gently until dissolved. Dilute 20 mL of 20X Wash Buffer Concentrate into deionized or distilled water to yield 400 mL of 1X Wash Solution.

9.5 **1X Biotinylated FGF basic Detection Antibody**

Briefly spin the Biotinylated anti-Human FGF basic vial prior to use. Add 100 µL of 1X Assay Diluent into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can either be stored at 4°C for 5 days or aliquoted and frozen at -20°C for 2 months). The detection antibody concentrate must be diluted 65-fold with 1X Assay Diluent prior to use in the Assay Procedure.

9.6 **1X HRP-Streptavidin Solution**

Briefly spin the 120X HRP-Streptavidin concentrate vial and pipette up and down to mix gently before use. HRP-Streptavidin concentrate must be diluted 120-fold with 1X Assay Diluent prior to use in the Assay Procedure.

For example: Briefly spin the vial and pipette up and down to mix gently. Add 100 µL of 120X HRP-Streptavidin concentrate into a tube with 12 mL 1X Assay Diluent to prepare a final 120 fold diluted 1X HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix well.

10. STANDARD PREPARATIONS

- Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards for every use.
- Standard (recombinant protein) should be stored at -20°C or -80°C (recommended at -80°C) after reconstitution.

10.1 Briefly spin the vial of FGF basic Standard. Prepare the 100 ng/mL FGF basic **Stock Standard** by adding 400 µL 1X Sample Diluent Buffer into the vial (see table below).

10.2 Dissolve the powder thoroughly by a gentle mix.

10.3 Label tubes #1-6.

10.4 Prepare **Standard #**1 by adding 100 µL 100 ng/mL **Stock Standard** to 900 µL Sample Diluent Buffer into tube #1. Mix thoroughly and gently.

10.5 Pipette 300 µL 1X Sample Diluent Buffer into each tube.

10.6 Prepare **Standard #**2 by transferring 200 µL from tube #1 to #2, mix thoroughly.

10.7 Prepare **Standard #**3 by transferring 200 µL from tube #2 to #3, mix thoroughly.

10.8 Using the table **below** as a guide, prepare further serial dilutions

10.9 1X Sample Diluent Buffer serves as the zero standard (0 pg/mL).

Standard Dilution Preparation Table

Standard #	Volume to Dilute (µL)	Diluent (µL)	Total Volume (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	100	900	1000	100,000	10,000
2	200	300	500	10,000	4,000
3	200	300	500	4,000	1,600
4	200	300	500	1,600	640
5	200	300	500	640	256
6	200	300	500	256	102.4
7	0	300	300	0	0

11. SAMPLE PREPARATION

General Sample Information:

- Tissue lysate and cell lysate sample may be diluted with 1X Sample Diluent Buffer.

12. 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 well strips should be returned to the plate packet and stored at 4°C.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Well effects have not been observed with this assay. Contents of each well can be recorded on the template sheet included in the Resources section.

13. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature (18 - 25°C) prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**

13.1. Add 100 µL of each standard (see Standard Preparation section 10) and sample into appropriate wells. Cover wells and incubate for 2.5 hours at room temperature or overnight at 4°C with gentle shaking. We recommend using 50-500 µg/mL of total protein for lysate sample. The amount of sample used depends on the abundance of target protein. More of the sample can be used if signals are too weak. If signals are too strong, the sample can be diluted further.

13.2. Discard the solution and wash 4 times with 1X Wash Solution. Wash by filling each well with 300 µL 1X Wash Solution using a multi-channel Pipette or auto washer. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining liquid by aspirating or decanting. Invert the plate and blot it against clean paper towels.

13.3. Add 100 µL of 1X Biotinylated FGF basic Detection Antibody (Reagent Preparation section 9) to each well. Incubate for 1 hour at room temperature with gentle shaking.

13.4. Discard the solution. Repeat the wash as in step 13.2.

13.5. Add 100 µL of 1X HRP-Streptavidin solution (see Reagent Preparation section 9) to each well. Incubate for 45 minutes at room temperature with gentle shaking.

13.6. Discard the solution. Repeat the wash as in step 13.2.

13.7. Add 100 µL of TMB One-Step Substrate Reagent to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.

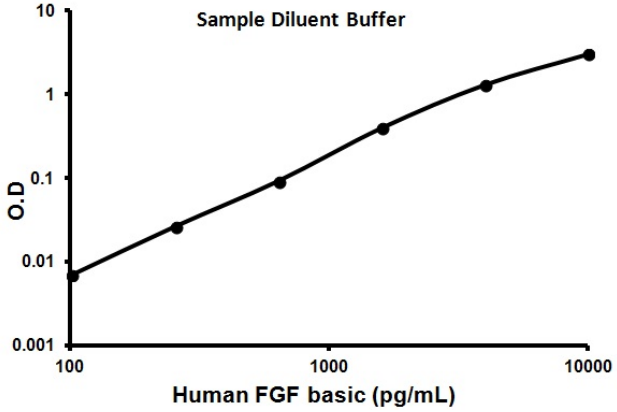
13.8. Add 50 µL of Stop Solution to each well. Read at 450 nm immediately.

14. CALCULATIONS

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

15. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.



Conc. (pg/mL)	O.D.
	Sample Diluent Buffer
102.4	0.007
256	0.027
640	0.093
1,600	0.397
4,000	1.302
10,000	3.023

16. TYPICAL SAMPLE VALUES

SENSITIVITY –

The minimum detectable dose of FGF basic is typically less than 50 pg/mL.

RECOVERY –

Recovery was determined by spiking Human FGF basic into normal Human tissue lysate and cell lysate. Mean recoveries are as follows:

Sample Type	Average % Recovery	Range (%)
Tissue Lysate	95.87	86-107
Cell Lysate	97.65	87-108

LINEARITY OF DILUTION -

Tissue Lysate	Average % Expected Value	Range (%)
1:2	97	89-107
1:4	96	88-106

Cell Lysate	Average % Expected Value	Range (%)
1:2	97	89-107
1:4	95	89-107

PRECISION –

	Intra-Assay	Inter-Assay
CV (%)	<10%	<12%

17. ASSAY SPECIFICITY

Cross Reactivity: This ELISA kit shows no cross-reactivity with the following cytokines tested: Human Angiogenin, BDNF, BLC, ENA-78, FGF-4, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12 p70, IL-12 p40, IL-13, IL-15, IL-309, IP-10, G-CSF, GM-CSF, IFN-γ, Leptin, MCP-1, MCP-2, MCP-3, MDC, MIP-1α, MIP-1 β, MIP-1δ, PARC, PDGF, RANTES, SCF, TARC, TGF-β, TIMP-1, TIMP-2, TNF-α, TNF-β, TPO, VEGF.

18. TROUBLESHOOTING

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standards dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; change to overnight standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store the reconstituted protein at -80°C, all other assay components 4°C. Keep substrate solution protected from light.

19. NOTES

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