Human FGF-b ELISA kit

Catalog Number: EK0480

For the quantitative determination of human FGF-b concentrations in cell culture supernates, serum, and plasma.

This instruction must be read in its entirety before using this product.

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

Contact information

Tel: 1-301-446-2499 Fax: 1-301-446-2413

Email: tech@signalwayantibody.com Web: www.sabbiotech.com

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for 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. Following incubation unbound samples are removed during a wash step, and then a detection antibody specific for FGF basic is added to the wells and binds to the combination of capture antibody- FGF basic in sample. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. Following incubation and wash steps a substrate is added. A coloured product is formed in proportion to the amount of FGF basic present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450nm. A standard curve is prepared from seven FGF basic standard dilutions and FGF basic sample concentration determined.

DETECTION RANGE

7.8 pg/ml - 1000 pg/ml

SENSITIVITY

The minimum detectable dose was 4pg/mL.

SPECIFICITY

This assay recognizes both natural and recombinant human FGF basic. The factors listed below were prepared at 50ng/ml in Standard /sample Diluent and assayed for cross-reactivity and no significant cross-reactivity or interference was observed.

Table 1: Factors assayed for cross-reactivity

Recombinant human	Recombinant mouse	Recombinant porcine
LIF	IL-1β	PDGF
EGF	EGF	TGF-β1
TGF-β1	GM-CSF	
TNF-α	TNF-α	
TNF-β		

PRECISION

The coefficient of variation of both intra-assay and inter-assay were lessthan 10%.

MATERIALS PROVIDED

1. Aluminium pouches with a Microwell Plate coated with antibody to human FGF basic (8 x 12)
2. 2 vials human FGF basic Standard lyophilized, 500 pg/ml upon reconstitution
3. 2 vials concentrated Biotin-Conjugate anti-human FGF basic antibody
4. 2 vials Streptavidin-HRP solution
5. 1 bottle Standard /sample Diluent
6. 1 bottle Biotin-Conjugate antibody Diluent
7. 1 bottle Streptavidin-HRP Diluent
8. 1 bottle Wash Buffer Concentrate 20x (PBS with 1% Tween-20)
9. 1 vial Substrate Solution
10. 1 vial Stop Solution
11. 4 pieces Adhesive Films
12. package insert

STORAGE

Table 2: Storage of the kit

<table>
<tr><td>Unopened Kit</td><td colspan="2">Store at 2 – 8℃. Do not use past kit expiration date.</td></tr>
<tr><td rowspan="9">Opened/ Reconstituted Reagents</td><td>Standard /sample Diluent</td><td rowspan="8">May be stored for up to 1 month at 2 – 8℃.**</td></tr>
<tr><td>Concentrated Biotin-Conjugate</td></tr>
<tr><td>Streptavidin-HRP solution</td></tr>
<tr><td>Biotin-Conjugate antibody Diluent</td></tr>
<tr><td>Streptavidin-HRP Diluent</td></tr>
<tr><td>Wash Buffer Concentrate 20x</td></tr>
<tr><td>Substrate Solution</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Standard</td><td>Aliquot and store for up to 1 month at -20℃. Avoid repeated freeze-thaw cycles. Diluted standard shall not be reused.</td></tr>
<tr><td>Microplate Wells</td><td>Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at2 – 8℃.**</td></tr>
</table>

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

THE REQUIRED ITEMS (not provided, but can help to buy)

1. Microplate reader (450nm).
2. Micro-pipette and tips: 0.5-10, 2-20, 20-200, 200-1000ul.
3. 37 ℃ incubator, double-distilled water or deionized water, coordinate paper, graduated cylinder.

PRECAUTIONS FOR USE

1. Store kit regents between 2℃and 8℃. After use all reagents should be immediately returned to cold storage(2℃ to 8℃).
2. Please perform simple centrifugation to collect the liquid before use.
3. To avoid cross contamination, please use disposable pipette tips.
4. The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material. Avoid contact of skin or mucous membranes with kit reagents or specimens. In the case of contact with skin or eyes wash immediately with water.

5. Use clean, dedicated reagent trays for dispensing the washing liquid, conjugate and substrate reagent. Mix all reagents and samples well before use.
6. After washing microtiter plate should be fully pat dried. Do not use absorbent paper directly into the enzyme reaction wells.
7. Do not mix or substitute reagents with those from other lots or other sources. Do not use kit reagents beyond expiration date on label.
8. Each sample, standard, blank and optional control samples should be assayed in duplicate or triplicate.
9. Adequate mixing is very important for good result. Use a mini-vortexerat the lowest frequency or Shake by hand at 10min interval when there is no vortexer.
10. Avoid microtiter plates drying during the operation.
11. Dilute samples at the appropriate multiple, and make the sample values fall within the standard curve. If samples generate values higher than the highest standard, dilute the samples and repeat the assay.
12. Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time and temperature, and kit age can cause variation in binding.

13. This method can effectively eliminate the interference of the soluble receptors, binding proteins and other factors in biological samples.

SAMPLE COLLECTION AND STORAGE

1. **Cell Culture Supernates** - Remove particulates by centrifugation.
2. **Serum** - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at approximately 1000 x g. Remove serum, avoid hemolysis and high blood lipid samples.
3. **Plasma** - Recommended EDTA as an anticoagulant in plasma. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection.
4. Assay immediately or aliquot and store samples at -20℃. Avoid repeated freeze-thaw cycles.
5. Dilute samples at the appropriate multiple (recommended to do pre-test to determine the dilution factor).

REAGENT PREPARATION

1. Bring all reagents to room temperature before use.
2. **Wash Buffer** - Dilute 10mL of Wash Buffer Concentrate into deionized or distilled water to prepare 200mL of Wash Buffer. If crystals have formed in the concentrate Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.
3. **Standard** - Reconstitute the Standard with 1.0mL of Standard /sample Diluent. This reconstitution produces a stock solution of 1000 pg /mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.
   Pipette 500μL of Standard/sample Diluent into the 500 pg/mL tube and the remaining tubes. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 500 pg/mL standard serves as the high standard. The Standard/ sample Diluent serves as the zero standard (0 pg/mL).
   If you do not run out of re-melting standard, store it at -20℃. Diluted standard shall not be reused.

4. Working solution of Biotin-Conjugate anti-human FGF basic antibody: Make a 1:100 dilution of the concentrated Biotin-Conjugate solution with the Biotin-Conjugate antibody Diluent in a clean plastic tube.
   The working solution should be used within one day after dilution.
5. Working solution of Streptavidin-HRP: Make a 1:100 dilution of the concentrated Streptavidin-HRP solution with the Streptavidin-HRP Diluent in a clean plastic tube.
   The working solution should be used within one day after dilution.

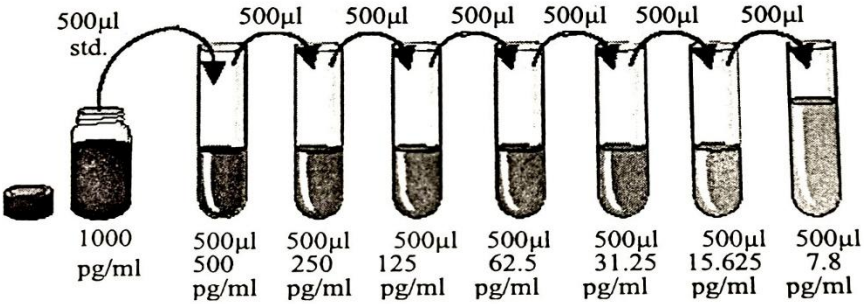

Figure 1: Preparation of FGF basic standard dilutions

GENERAL ELISA PROTOCOL

1. Prepare all reagents and working standards as directed in the previous sections.
2. Determine the number of microwell strips required to test the desired number of samples plus appropriate number of wells needed for running blanks and standards. Remove extra microwell strips from holder and store in foil bag with the desiccant provided at 2-8℃ sealed tightly.
3. Add 100μL of Standard, control, or sample, per well. Cover with the adhesive strip provided. Incubate for 1.5 hours at 37℃.
4. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (350μL) using a squirt bottle, manifold dispenser or auto-washer. 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.

5. Add 100 μL of the working solution of Biotin-Conjugate to each well. Cover with a new adhesive strip and incubate 1 hours at 37℃.
6. Repeat the aspiration/wash as in step 4.
7. Add 100 μL of the working solution of Streptavidin-HRP to each well. Cover with a new adhesive strip and incubate for 30 minutes at 37℃. Avoid placing the plate in direct light.
8. Repeat the aspiration/wash as in step 4.
9. Add 100 μL of Substrate Solution to each well. Incubate for 10-20 minutes at 37℃. Avoid placing the plate in direct light.
10. Add 100 μL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
11. Determine the optical density of each well immediately, using a microplate reader set to 450 nm.(optionally 630nm as the reference wave length; 610-650nm is acceptable)

ASSAY PROCEDURE SUMMARY

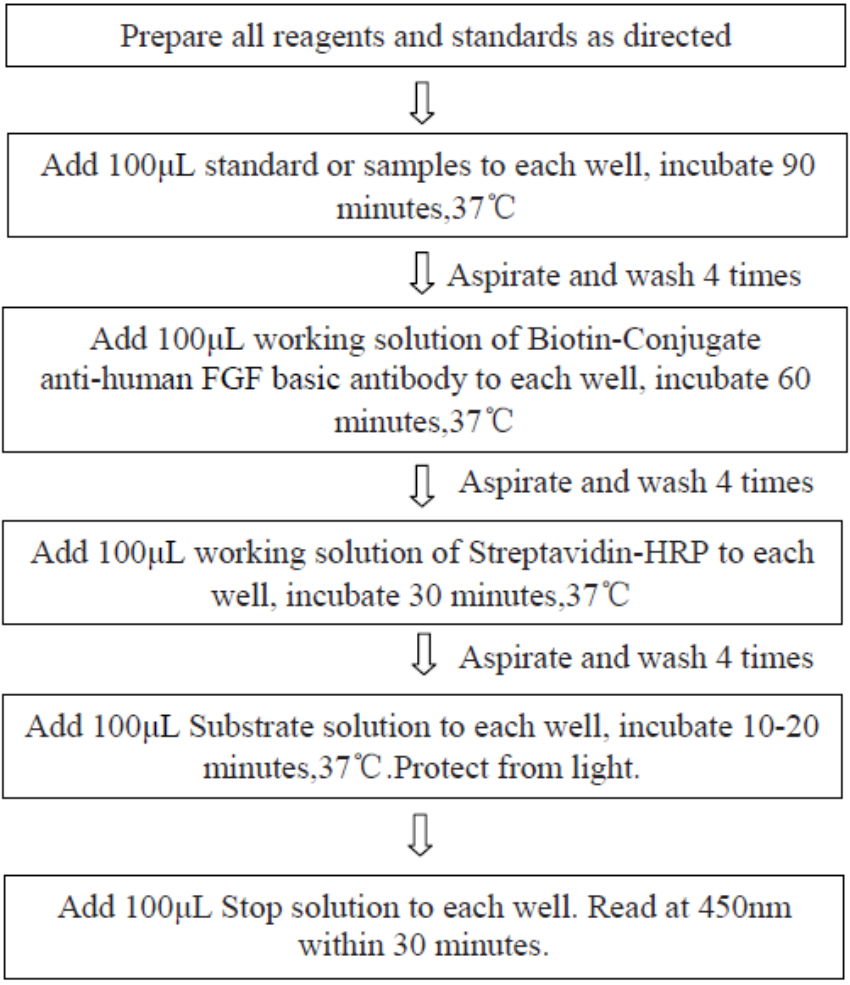

Figure 2: Assay procedure summary

TECHNICAL HINTS

1. When mixing or reconstituting protein solutions, always avoid foaming.
2. 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.
3. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
4. Substrate Solution should remain colorless until added to the plate. Stop Solution should be added to the plate in the same order as the Substrate Solution. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
5. A standard curve should be generated for each set of samples assayed. According to the content of tested factors in the sample, appropriate diluted or concentrated samples, it is best to do pre-experiment.

CALCULATION OF RESULTS

1. Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density.
2. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) 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 the graph.
3. The data may be linearized by plotting the log of the FGF basic concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
4. This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Table 3: Typical data using the FGF basic ELISA (Measuring wavelength: 450nm, Reference wavelength: 630nm)

Standared(pg/ml)	OD.	OD.	Average	Corrected
0	0.052	0.049	0.051	——
7.8	0.078	0.073	0.076	0.025
15.625	0.083	0.085	0.084	0.033
31.25	0.149	0.145	0.147	0.096
62.5	0.247	0.250	0.249	0.198
125	0.548	0.542	0.545	0.494
250	1.194	1.189	1.192	1.141
500	2.067	2.059	2.063	2.012

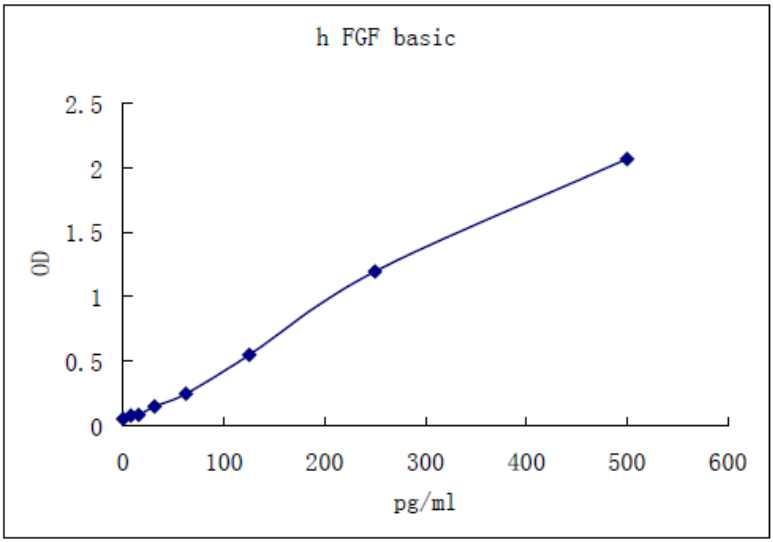

Figure 3: Representative standard curve for FGF basic ELISA. FGF basic was diluted in serial two-fold steps in Sample Diluent.

Do not use this standard curve to derive test results. A standard curve must be run for each group of microwell strips assayed.

BACKGROUND

FGF basic, also called FGF-2 (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). Expression of FGF basic is nearly ubiquitous. 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. 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).

REFERENCES

1. Mohammadi, M. et al. (2005) Cytokine Growth Factor Rev. 16:107.
2. Presta, M. et al. (2005) Cytokine Growth Factor Rev. 16:159.
3. Reuss, B. et al. (2003) Cell Tissue Res. 313:139.
4. Su, N. et al. (2008) Front. Biosci. 13:2842.
5. Grose, R. and C. Dickson (2005) Cytokine Growth Factor Rev. 16:179.
6. Abraham, J.A. et al. (1986) EMBO J. 5:2523.
7. Kurokawa, T. et al. (1987) FEBS Lett. 213:189.
8. Claus, P. et al. (2003) J. Biol. Chem. 278:479.
9. Quarto, N. et al. (2005) Gene 356:49.
10. Kardami, E. et al. (2004) Cardiovasc. Res. 63:458.
11. Dono, R. et al. (1998) EMBO J. 17:4213.
12. Rosenblatt-Velin, N. et al. (2005) J. Clin. Invest. 115:1724.
13. Pellieux, C. et al. (2001) J. Clin. Invest. 108:1843.
14. Montero, A. et al. (2000) J. Clin. Invest. 105:1085.
15. Miller, D. et al. (2000) Mol. Cell. Biol. 20:2260.
16. Coffin, J.D. et al. (1995) Mol. Biol. Cell 6:1861.
17. Sobue, T. et al. (2005) J. Cell. Biochem. 95:83.