Human FGF acidic ELISA Kit

Enzyme Immunoassay for the quantification of Human FGF acidic in serum, plasma, cell culture supernatants

Catalog number: ARG81255

Package: 96 wells

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

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INTRODUCTION

FGF acidic is a member of the fibroblast growth factor (FGF) family. FGF family members possess broad mitogenic and cell survival activities, and are involved in a variety of biological processes, including embryonic development, cell growth, morphogenesis, tissue repair, tumor growth and invasion. This protein functions as a modifier of endothelial cell migration and proliferation, as well as an angiogenic factor. It acts as a mitogen for a variety of mesoderm- and neuroectoderm-derived cells in vitro, thus is thought to be involved in organogenesis. Multiple alternatively spliced variants encoding different isoforms have been described. [provided by RefSeq, Jan 2009]
FGF acidic plays an important role in the regulation of cell survival, cell division, angiogenesis, cell differentiation and cell migration. Functions as potent mitogen in vitro. [UniProt]

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for FGF acidic has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any FGF acidic present is bound by the immobilized antibody. After washing away any unbound substances, a biotin-conjugated antibody specific for FGF acidic is added to each well and incubate. Following a washing to remove unbound substances, streptavidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of FGF acidic bound in the initial step. The color development is stopped by the addition of acid and the intensity of

the color is measured at a wavelength of 450nm ±2nm.The concentration of FGF acidic in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8°C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	8 X 12 strips	4°C
Standard (Lyophilized)	2 X 8 ng/vial	4°C, store at -20°C after reconstitution
Standard/Sample diluent	16 ml	4°C
Antibody conjugate concentrate	2 vials (60 µl)	4°C
Antibody diluent buffer	16 ml	4°C
HRP-Streptavidin concentrate	2 vials (60 µl)	4°C (Protect from light)
HRP-Streptavidin diluent buffer	16 ml	4°C
20X Wash buffer	25 ml	4°C
TMB substrate	12 ml	4°C (Protect from light)
STOP solution	12 ml	4°C
Plate sealer	4 strips	Room temperature

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm (optional: read at 610-650 nm as the reference wave length)
- Pipettes and pipette tips
- Deionized or distilled water
- 37°C oven or incubator
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- If crystals are observed in the 20X Wash buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- All materials should be equilibrated to room temperature (RT, 20-25°C) 20 min before use.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Mix the contents of the microplate wells thoroughly by microplate shaker for 1 min or gently tap the plate to ensure good test results. Please mix carefully to avoid well-to-well contamination. Do not reuse microwells.

- The TMB Color developing agent should be colorless and transparent before using.
- Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
- Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
- Avoid using reagents from different batches.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

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

Cell Culture Supernatants - Remove particulates by centrifugation and aliquot & store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma on ice using EDTA as an anticoagulant. Centrifuge (1000 x g) for 15 minutes at 2-8°C within 30 minutes of collection. Collect the

supernatants and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

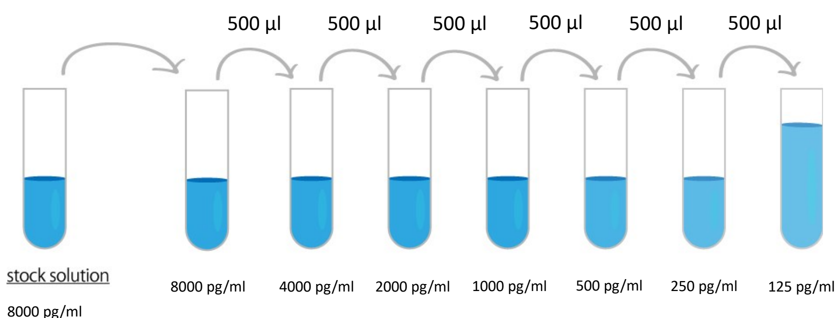
- **1X Wash buffer**: Dilute 20X Wash buffer into distilled water to yield 1X Wash buffer. (E.g. 25 ml of 20X Wash buffer + 475 ml of distilled water) The diluted Wash buffer is stable for 4 weeks at 2°C to 8°C.
- **1X Antibody conjugate**: 20 minutes before use, dilute 100X antibody conjugate concentrate into Antibody diluent buffer to yield 1X detection antibody solution.
- **1X HRP-Streptavidin Solution**: 20 minutes before use, dilute 100X HRP-Streptavidin concentrate solution into HRP-Streptavidin diluent buffer to yield 1X HRP-Streptavidin Solution buffer. Keep diluted HRP-Streptavidin Solution in dark before use.
- **Sample**: If the initial assay found samples contain FGF acidic higher than the highest standard, the samples can be diluted with Standard/Sample diluent and then re-assay the samples. For the calculation of the concentrations this dilution factor has to be taken into account.

  Note: The normal human serum/plasma samples are suggested to make a 1:2 dilution with Standard/Sample diluent before assay.

  (It is recommended to do pre-test to determine the suitable dilution factor).

- **Standards**: Reconstitute the standard with 1 ml Standard/Sample diluent to yield a stock concentration of 8000 pg/ml. Allow the stock standard to sit for at least 15 minutes with gentle agitation to make sure the standard is dissolved completely before making serial dilutions. The Standard/ Sample diluent serves as zero standard (0 pg/ml), and the rest of the standard serial dilution can be diluted with Standard/ Sample diluent as according to the suggested concentration below: 8000 pg/ml, 4000 pg/ml, 2000 pg/ml, 1000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml.

  Note: The reconstitute stock standard can be aliquoted and stored at ≤ -20°C up to a month. DO NOT reused the diluted standards.



ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-25°C) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add **100 µl** of **standards**, **samples** and **zero controls** (Standard/Sample diluent) into wells. Incubate for **1.5 h** at **37°C**.
3. Aspirate each well and wash, repeating the process 3 times for a total 4 washes. Wash by filling each well with 1× Wash Buffer (350 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
4. Add **100 µl** of **1X Antibody conjugate** into each well. Cover wells and incubate for **1 hour** at **37°C**.
5. Aspirate each well and wash as step 3.
6. Add **100 µl** of **1X HRP-Streptavidin solution** to each well. Cover wells and incubate for **30 minutes** at **37°C** in dark.
7. Aspirate each well and wash as step 3.
8. Add **100 µl** of **TMB Reagent** to each well. Incubate for **10-20 minutes** at **37°C** in dark.
9. Add **100 µl** of **Stop Solution** to each well. Gently tap the plate to ensure thorough mixing. The color of the solution should change from blue to

yellow.

10. Read the OD with a microplate reader at 450nm immediately. (optional: read at 610-650 nm as the reference wave length) It is recommended read the absorbance within 30 min after adding Stop solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using linear or semi-log graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

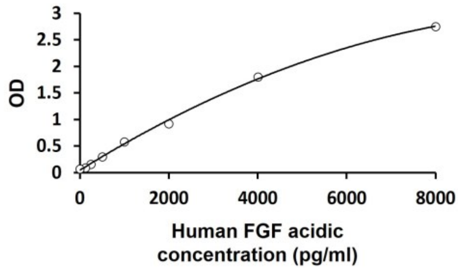
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)

6. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of Human FGF acidic ranged from 125-8000 pg/ml. The mean MDD was 60 pg/ml.

Specificity

This assay recognizes natural and recombinant Human FGF acidic. No significant cross-reactivity or interference with the factors below was observed: 50 ng/ ml of recombinant proteins:

Human: FGF basic, FGF-BP, FGF R1 alpha (IIIb)/Fc Chimera, FGF R1 alpha (IIIc)/Fc Chimera, FGF R1 beta (IIIb)/Fc Chimera, FGF R1 beta (IIIc)/Fc Chimera, FGF R2 alpha (IIIb)/Fc Chimera, FGF R2 alpha (IIIc)/Fc Chimera, FGF R2 beta (IIIb)/Fc Chimera, FGF R2 beta (IIIc)/Fc Chimera, FGF R3 (IIIb)/Fc Chimera, FGF R3 (IIIc)/Fc Chimera, FGF R4/Fc Chimera, S100A13

Mouse: FGF-8b, Flt-3 Ligand, G-CSF, GM-CSF, M-CSF, PlGF-2, VEGF120

Rat: GM-CSF, beta-NGF, PDGF-BB

Intra-assay and Inter-assay precision

The CV values of both intra and inter precision fall below 10%.