Bovine FGF Acidic ELISA Kit

Acidic fibroblast growth factor known as FGF acidic, also known as FGF-1, ECGF, and HBGF-1, is a mitogenic peptide that is produced by multiple cell types and stimulates the proliferation of cells of mesodermal, ectodermal, and endodermal origin. FGF acidic is involved in wound repair, angiogenesis, and development. FGF acidic is secreted from cells via an endoplasmic reticulum/Golgi independent mechanism (1, 2). The ability of FGF acidic to bind to heparin sulfate is required for its ability to interact with FGF receptors and induce signaling (1-4). There are four distinct FGF receptors and each has multiple splice variants. FGF acidic binds with high affinity to many, but not all, FGFRs. Signaling cascades activated through FGF basic binding to FGFR include the ras-raf-MAPK, PLCγ/PKC, and PI3K/Akt pathways.

References

1. Prudovsky, I. et al. (2003) J Cell Sci 116, 4871.
2. Powers, C.J. et al. (2000) Endocr Relat Cancer 7, 165.
3. Mohammadi, M. et al. (2005) Curr Opin Struct Biol 15, 506.
4. Ornitz, D.M. and Itoh, N. (2001) Genome Biol 2, REVIEWS3005.

PRINCIPLE OF THE ASSAY

This kit is for quantification of FGF acidic in cow, cattle, bull. This is a shorter ELISA assay that reduces time to 50% compared to the conventional method, and the entire assay only takes 3 hours. This assay employs the quantitative sandwich enzyme immunoassay technique and uses biotin-streptavidin chemistry to improve the performance and the sensitivity of the assays. An antibody specific for bovine FGF acidic has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any FGF acidic present is bound by the immobilized antibody. After washing away any unbound substances, a detection antibody specific for bovine FGF acidic is added to the wells. Following wash to remove any unbound antibody reagent, a detection reagent is added. After intensive wash a substrate solution 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 and the intensity of the color is measured.

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

Storage

Store the standard and detection antibody at 4°C or -20°C, 10 x reagent diluents and the antibody plate at -20°C and the rest of the kit at 4°C. The kit should be used in 3 months.

MATERIALS PROVIDED

Description	Quantity	Description	Quantity	Description	Quantity
Antibody Precoated Plate	1	20 x PBS	1	Substrate Solution	1
Detection Antibody	1	20 x Wash Buffer	1	Stop Solution	1
Detection Agent	1	10 x Reagent Diluent	1	DataSheet/Manual	1
Standard	3	20 x Standard/Sample Diluent	1	96-well plate sheet	1

Bring all reagents to room temperature before use.
1 x **96-well Plate precoated with Bovine FGF Acidic antibody-**Store at 4°C or -20°C upon receipt.

Bovine FGF Acidic Detection Antibody (1 vial) – The lyophilized Detection Antibody should be stored at 4°C or -20°C in a manual defrost freezer for up to 3 months, if not used immediately. Centrifuge for 1 min at 6000 x g to bring down the material prior to open the vial. The vial contains sufficient Detection Antibody for a 96-well plate. Add 100 µL of sterile 1 x PBS to the antibody vial and vortex for 20 sec and allow it to sit for 5 min prior to open the vial. If the entire 96-well plate is used, take 100 µL of detection antibody to 9.9 mL 1 x Reagent Diluent. If the partial antibody is used store the rest at -20°C until use.

Bovine FGF Acidic Standard (3 vials) – The lyophilized Bovine FGF Acidic Standard has 3 vials. Each vial contains the standard sufficient for generating a standard curve. The non-reconstituted standard can be stored at 4°C or -20°C for up to 3 months if not used immediately. Centrifuge for 1 min at 6000 x g to bring down the material prior to open the vial. Add 500 µL of 1 x Standard/Sample Diluent to one Standard vial to make the high standard concentration of 3000 pg /ml. Vortex for 20 sec and allow it to sit for 5 min prior to use. A seven point standard curve is generated using 2-fold serial dilutions in 1 x Standard/Sample Diluent, each in duplicate, vortex for 20 sec for each of dilution steps.

Detection Agent (1 vial) – Centrifuge for 1 min at 6000 x g to bring down the material prior to open the vial. The vial contains sufficient Detection Agent for a 96-well plate. Add 100 µL of sterile 1 x PBS to a vial and vortex 30 sec and allow it to sit for 5 min. Make 1:100 dilutions in Reagent Diluent. If the entire 96-well plate is used, add 100 µL of Detection Agent to 9.9 mL of 1 x Reagent Diluent prior to the assay. The rest of undiluted Detection Reagent can be stored at 4°C for up to 6 months. DO NOT FREEZE.

20 x PBS, pH 7.3, 30 mL- Dilute to 1 x PBS with deionized distilled water and mix well prior to use.
20 x Wash Buffer, 20 mL- Dilute to 1 x Wash Buffer with 1 x PBS prior to use.
10 x Reagent Diluent– Add 3 mL of sterile 1 x PBS to make 10 x Reagent Diluent and vortex for 1 min and allow it to sit for 15 min to completely dissolve. Store at -20 °C. Prior to use dilute to 1 x Reagent Diluent with 1 x PBS.
20 x Standard/Sample Diluent, 10 mL- Dilute to 1 x Standard/Sample Diluent with 1 x PBS.
Substrate Solution, 10 mL.
Stop Solution, 5 mL.

Assay Procedure

1. Lift the plate cover from the top left corner and cover the wells that are not used. Vortex briefly the samples and the standards prior to the assay. Add 100 µL of sample (such as plasma or serum) or standard to each well and use duplicate for samples and standards, cover the 96-well plate and incubate 1 hour at room temperature.
2. Aspirate each well and wash with 1 x Wash Buffer, repeating the process two times for a total of three washes. Wash by filling each well with 1 x Wash Buffer (300 µL) using a multi-channel pipette, manifold dispenser or auto-washer. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or by inverting the plate and blotting it against clean paper towels.
3. Add 100 µL of the working dilution of the Detection Antibody to each well. Cover the plate and incubate 1 hour at room temperature.
4. Repeat the aspiration/wash as in step 2.
5. Add 100 µL of the working dilution of Detection Agent to each well. Cover the plate and incubate for 20 minutes at room temperature. Avoid placing the plate in direct light.
6. Repeat the aspiration/wash as in step 2.
7. Add 100 µL of Substrate Solution to each well. Incubate for 5-10 minutes at room temperature. Avoid placing the plate in direct light.
8. Add 50 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well immediately, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not 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.

Precaution and Technical Notes

1. A standard curve should be generated for each set of samples assayed. Thorough mixing of the standards at each of dilution steps is crucial to ensure a normal standard curve.
2. If FGF Acidic exceeds the upper limit of the detection, the sample needs to be diluted with the 1 x Wash Buffer. The dilution factor must be used for calculation of the concentration.
3. Detection Agent contains enzyme, DO NOT mass up with Detection Antibody.
4. The Stop Solution is an acid solution, handle with caution.
5. This kit should not be used beyond the expiration date on the label.
6. A thorough and consistent wash technique is essential for proper assay performance. Wash Buffer should be dispensed forcefully and removed completely from the wells by aspiration or decanting. Remove any remaining Wash Buffer by aspiration or by inverting the plate and blotting it against clean paper towels.
7. Use a fresh reagent reservoir and pipette tips for each step.
8. It is recommended that all standards and samples be assayed in duplicate.
9. Avoid microbial contamination of reagents and buffers. This may interfere with the sensitivity of the assay.

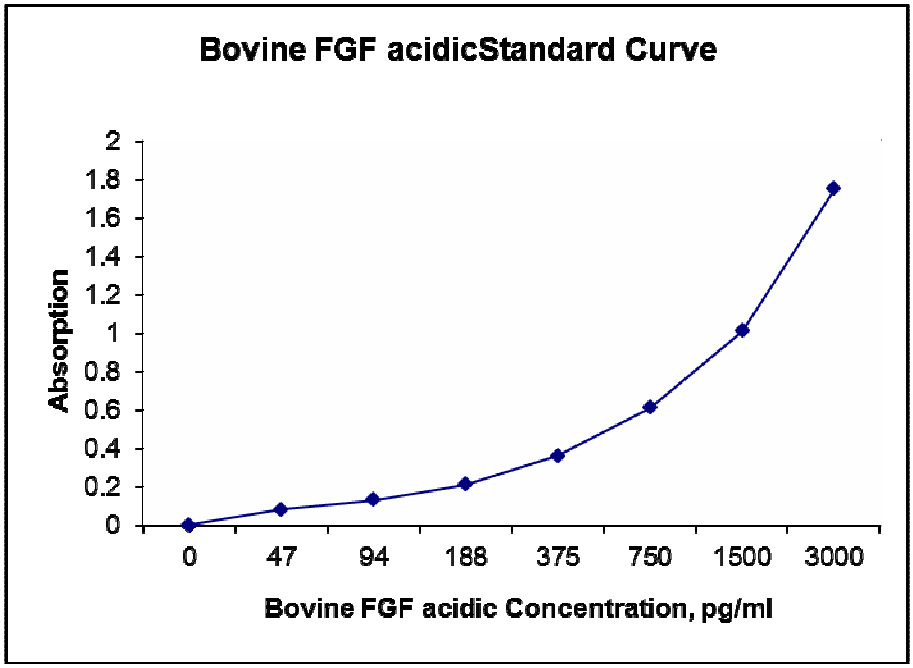
Calculation of Results

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

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. The data may be linearized by plotting the log of the FGF Acidic 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.

The Standard Curve

The graph below represents typical data generated when using this bovine FGF Acidic ELISA Kit. The standard curve was calculated using a computer generated 4-PL curve-fit. For this case, a Bio-Rad iMark™ Microplate Reader and a Microplate Manager 6 Software were used to generate this curve. The correlation coefficient (r^2) is 0.999-1.000.



Specificity

The following recombinant Bovine proteins prepared at 10 ng/ml were tested and exhibited no cross-reactivity or interference.
ApoAI, BMP1, BMP2, BMP4, BMP7, HGF, HSP27, IL-1β, IL-1RA, IL-2, IL-2R, IL-5, IL-6, IL-6R, IL-8, IL-10, IL-12, IL-13, IL-15, IL-17C, IL-23, IFNγ, MMP-2, MMP-9, PDGF-AA, PDGF-BB, PDGF-AB, TGFβ1, TGFβ2, TGFβ3, TLR1, TLR2, TLR3, TNF-α, TNF RI, TNF RII.

Calibration

This kit is calibrated against a highly purified CHO cell-expressed recombinant bovine FGF Acidic.

Detection Range

47-3000 pg/ml

Assay Sensitivity

7 pg/ml

Assay Precision

Intra-Assay %CV: 4; Inter-Assay %CV: 9

For Research Use Only

Related products

1. 20 x Sample Diluent
2. 20 x PBS
3. 10 x ELISA Wash Buffer
4. 10 x ELISA Reagent Diluent
5. Universal Blocking Buffer
6. 2 x Recombinant Protein Stabilizer
7. 5 x Recombinant Protein Stabilizer
8. ELISA G-Blue Substrate Solution
9. Bovine FGF Acidic Standard
10. Bovine FGF Acidic detection antibody