Nori® Sheep FGF Acidic ELISA Kit -2 Plates DataSheet

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 sheep. It 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 sheep 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 sheep 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 at 4°C. The kit should be used in 3 months.

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MATERIALS PROVIDED

Description	Quantity	Description	Quantity	Description	Quantity
Antibody Precoated Plate	2	20 x PBS	2	Substrate Solution	1
Detection Antibody	2	40 x Assay Buffer	1	Stop Solution	1
Conjugate	1	2 x Reagent Diluent	1	DataSheet/Manual	1
Standard	4	MSDS/CoA	1	96-well plate sheet	2

Bring all reagents to room temperature before use.

Reagent Preparations

Sheep FGF Acidic Detection Antibody (2 vials) – 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. Each 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 10.5 mL of 1 x Reagent Diluent to make working dilution of Detection Antibody and mix thoroughly prior to the assay. If the partial antibody is used store the rest at -20°C until use.

Sheep FGF Acidic Standard (4 vials) – The lyophilized Sheep FGF Acidic Standard has 4 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 Assay Buffer to one Standard vial to make the high standard concentration of 2,400 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 Assay Buffer, each in duplicate, vortex for 20 sec for each of dilution steps.

Conjugate (106 µL) – Centrifuge for 1 min at 6000 x g to bring down the material prior to open the vial. The vial contains 106 µL Conjugate sufficient for two 96-well plates. If the volume is less than 106 µL, add sterile 1 x PBS to reach 106 µL and vortex 10 sec. Make 1:200 dilutions in 1 x Reagent Diluent. If the entire 96-well plate is used, add 53 µL of Conjugate to 10 mL of 1 x Reagent Diluent to make **working dilution of Conjugate** prior to the assay. The rest of undiluted Conjugate can be stored at 4°C for up to 3 months. DO NOT FREEZE.

20 x PBS, pH 7.3, 2 x 30 mL- Dilute to 1 x PBS with deionized distilled water and mix well prior to use.
80 x Assay Buffer, 10 mL- Dilute to 1 x Assay Buffer with 1 x PBS prior to use.
2 x Reagent Diluent, 21 mL- Dilute to 1 x Reagent Diluent with 1 x PBS prior to use.
Substrate Solution, 21 mL.
Stop Solution, 11 mL.

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Assay Procedure

1. Lift the plate cover at the top left corner and cover the wells that are not used. Vortex the samples prior to the assay. Add 100 µL of **sample** (such as plasma or serum) or **standards** per well and use duplicate wells for each sample. Cover the 96-well plate and incubate 1 hour at room temperature.
2. Aspirate each well and wash with **1 x Assay Buffer**, repeating the process two times for a total of three washes. Wash by filling each well with 1 x Assay 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 Assay Buffer by aspirating or by inverting the plate and blotting it against clean paper towels.
3. Add 100 µL of **the working dilution of 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 Conjugate** 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 10-20 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. It is critical to follow the procedure step by step otherwise appropriate color development may not occur as expected.
2. Thorough mixing of the standards at each step of dilutions is critical to ensure a normal standard curve. A standard curve should be generated for each set of samples assayed.
3. Plasma or serum sample should be diluted with equal volume of 1 x Assay Buffer and vortex for 1 min prior to assay. If the OD value still exceeds the upper limit of the standard curve, further dilution is recommended till it falls in the detection range and the dilution factor must be used for calculation of the concentration.
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.
7. It is recommended that all standards and samples be assayed in duplicate.
8. Avoid microbial contamination of reagents and buffers. This may interfere with the sensitivity of the assay.

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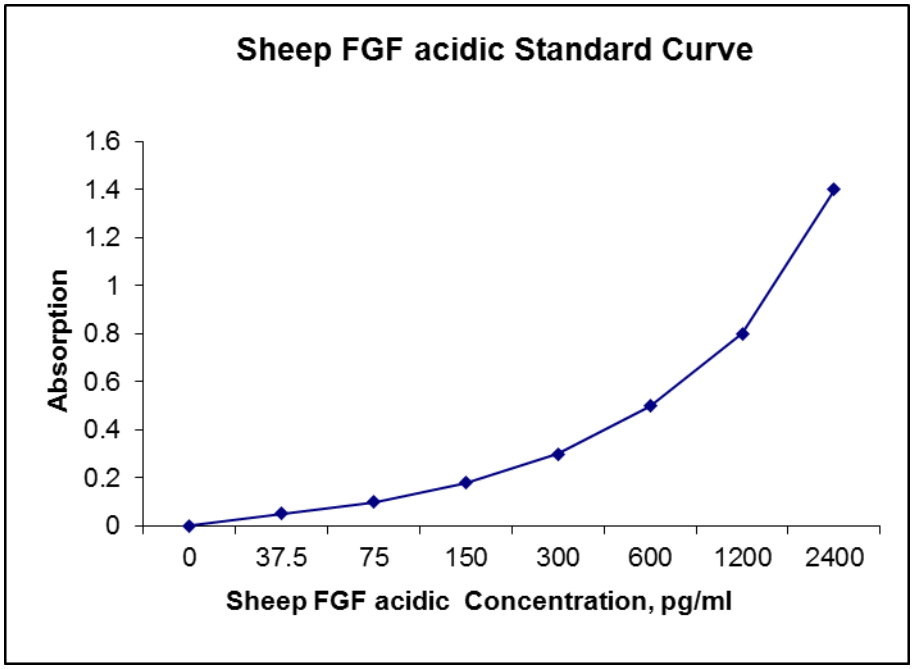
Calculation of Results

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 sheep 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.



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Specificity

The following recombinant Sheep 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 sheep FGF Acidic.

Detection Range

37-2,400 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, GR103058
2. 20 x PBS, Cat. GR103004-20
3. 10 x ELISA Assay Buffer, Cat. GR103028
4. 10 x ELISA Reagent Diluent, Cat. GR103055
5. Universal Blocking Buffer, Cat. GR103005
6. 2 x Recombinant Protein Stabilizer, Cat. GR103014-2
7. 5 x Recombinant Protein Stabilizer, Cat. GR103014-5
8. ELISA G-Blue Substrate Solution, Cat. GR103021
9. Sheep FGF Acidic Standard
10. Sheep FGF Acidic detection antibody

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Troubleshooting Guide

Problem	Possible causes	Solution
Poor standard curve	• Inaccurate pipetting • Improper standard curve	• Check pipettes • Check and use the correct dilution buffer • Vortex 30 sec for each of standard dilution steps
Low signal	• Improper preparation of standard, samples, detection antibody, and/or conjugate • Too brief incubation times • Inadequate reagent volume or improper dilution	• Briefly spin down vials before opening. Reconstitute the powder thoroughly. • Ensure sufficient incubation time. • Check pipettes and ensure correct preparation.
Large CV	• Inaccurate pipetting and mixing • Improper standard/sample dilutions. • Air bubbles in wells.	• Check pipettes and ensure thorough mixing. • Use the correct dilution buffers • Remove bubbles in wells.
High background	• Plate is insufficiently washed. • Contaminated Assay Buffer	• Review the datasheet for proper wash. If using a plate washer, ensure that all ports are unobstructed. • Make fresh Assay Buffer
No signal detected	• The procedure was misconducted.	• Ensure the step-by-step protocol was correctly followed and no misstep was conducted.
Low sensitivity	• Improper storage of the ELISA kit • Stop solution	• Store standards and detection antibody at -20°C after reconstitution, others at 4°C. Keep substrate protected from light. • Adding stop solution to each well before reading plate