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Mouse FGF2(Fibroblast Growth Factor 2, Basic) ELISA Kit

Cat# E5046
store at at -20°C or -80°C

INFORMATION

Size	96T
Sensitivity	4.41 pg/mL
Reactivity	Mouse
Detection range	15.63-1000 pg/mL
Specificity	This assay has high sensitivity and excellent specificity for detection of Mouse FGF2. No significant cross-reactivity or interference between Mouse FGF2 and analogues was observed. Please refer to the outer packaging label of the kit for the specific shelf life.
Special Explanation	1. Store kit at 4°C immediately upon receipt. 2. Do not use the kit after the expiration date. 3. Please check whether all components are complete after opening the package.All kit components have been formulated and quality control tested to function successfully as a kit. Do not mix or substitute reagents or materials from other kit, performance cannot be guaranteed if utilized separately or substituted.
Materials Required, Not Supplied	1. Microplate reader capable of measuring absorbance at 450 ± 10 nm. 2. High-speed centrifuge. 3. Electro-heating standing-temperature cultivator. 4. Absorbent paper. 5. Distilled or deionized water. 6. Single or multi-channel pipettes with high precision and disposable tips. 7. Precision pipettes to deliver 2μL to 1mL volumes.
Safety notes	1. This kit is sold for lab research and development use only and not for use in humans or animals. 2. Reagents should be treated as hazardous substances and should be handled with care and disposedof properly. 3. Gloves, lab coat, and protective eyewear should always be worn,Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.
Test Principle	This assay employs the competitive inhibition enzyme immunoassay technique. The microtiter plate provided in this kit has been pre-coated with Fibroblast Growth Factor 2, Basic(FGF2) protein. Standards or samples are added to the appropriate microtiter plate wells then with a biotin-conjugated antibody specific to Fibroblast Growth Factor 2, Basic(FGF2). Next,Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After TMB substrate solution is added. The enzyme-substrate reaction is terminated by the addition of sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450nm ± 10nm. The concentration of Fibroblast Growth Factor 2, Basic(FGF2) in the samples is then

	determined by comparing the OD of the samples to the standard curve.
Sample collection and storage	**Serum** - Use a serum separator tube and allow samples to clot for two hours at room temperature or overnight at 4°C before centrifugation for 20 minutes at approximately 1000×g. Assay freshly prepared serum immediately or store samples in aliquot at -20°C or -80°C for later use .Avoid repeated freeze/thaw cycles Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2-8°C within 30 minutes of collection. Remove plasma and assay immediately or store samples in aliquot at -20°C or -80°C for later use .Avoid repeated freeze/thaw cycles. **Tissue homogenates** -The preparation of tissue homogenates will vary depending upon tissue type. 1. Tissues were rinsed in ice-cold PBS to remove excess blood thoroughly and weighed before homogenization. 2. Minced the tissues to small pieces and homogenized them in fresh lysis buffer (different lysis buffer needs to be chosen based on subcellular location of the target protein) (w:v=1:9, e.g. 900 µL lysis buffer is added in 100 mg tissue sample) with a glass homogenizer on ice (Micro Tissue Grinders woks, too). 3. The resulting suspension was sonicated with an ultrasonic cell disrupter till the solution is clarified. 4. Then, the homogenates were centrifuged for 5 minutes at 10000×g. Collection the supernatant and assay immediately or aliquot and store at ≤-20°C. **Cell Lysates** - Cells need to be lysed before assaying according to the following directions. 1. Adherent cells should be washed by cold PBS gently, and then detached with trypsin, and collected by centrifugation at 1000×g for 5 minutes (suspension cells can be collected by centrifugation directly). 2. Wash cells three times in cold PBS. 3. Cells were then resuspended in fresh lysis buffer with concentration of 107 cells/mL. If it is necessary, the cells could be subjected to ultrasonication till the solution is clarified. 4. Centrifuge at 1500×g for 10 minutes at 2-8°C to remove cellular debris. Assay immediately or aliquot and store at ≤-20°C. **Urine** -Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter, assay immediately or aliquot and store at ≤-20°C. **Saliva** - Collect saliva using a collection device or equivalent. Centrifuge samples for 15 minutes at 1,000×g at 2-8°C. Remove particulates and assay immediately or store samples in aliquot at ≤-20°C.Avoid repeated freeze/thaw cycles. Cell culture supernatants and other biological fluids - Centrifuge samples for 20 minutes at 1000×g. Collect the supernatant and assay immediately or store samples in aliquot at -20°C or -80°C for later use.Avoid repeated freeze/thaw cycles.
Note	1. Samples to be used within 5 days may be stored at 4°C, otherwise samples must be stored at -20°C(≤1 month) or -80°C (≤2 months) to avoid loss of bioactivity and contamination. Avoid repeated freeze/thaw cycles. 2. Sample hemolysis will influence the result, so hemolytic specimen should not

	be used. 3. When performing the assay,bring samples to room temperature. 4. If the concentration of the test material in your sample is higher than that of the standard product,please make the appropriate multiple dilution according to the actual situation (it is recommended to do preliminary experiment to determine the dilution ratio.
Summary	1. After the kit is equilibrated at room temperature, add 50µL of standard working solution or 50µL of sample to each well, immediately add 50µL of Biotinylated-antigen working solution to each well, mix well, incubate at 37°C for 60min . 2. Discard the liquid in the plate, add 200µL Wash Buffer to each well, and wash the plate 3 times. After drying, add 100µL Streptavdin-HRP working solution to each well, incubate at 37°C for 60min . 3. Discard the liquid in the plate, add 200µL Wash Buffer to each well, and wash the plate 5 times. After spin-drying, add 90µL TMB to each well, incubate at 37°C for 20min. 4. Add 50µL stop solution to each well, read plate at 450nm immediately, calculation of the results.
Reagent preparation	1. Bring all kit components and samples to room temperature (18-25°C) before use. 2. If the kit will not be used up in one time, please only take out strips and reagents for present experiment, and save the remaining strips and reagents as specified. 3. Dilute the 25x wash buffer into 1x working concentration with double steaming water. 4. Biotinylated-Conjugate (1x) - Centrifuge the vial before opening. Biotinylated-Conjugate requires a 100-fold dilution. A suggested 100-fold dilution is 10 µL of Biotinylated-Conjugate with 990 µL of Biotinylated-Conjugate Diluent. 5. Standard -Reconstitute the Standard with 1.0mL of Standard Diluent, kept for 10 minutes at room temperature, shake gently(not to foam). The concentration of the standard in the stock solution is 1000 pg/mL. Please prepare 7 tubes containing 0.5mL Standard Diluent and use the diluted standard to produce a double dilution series according to the picture shown below. Mix each tube thoroughly before the next transfer. Set up 7 points of diluted standard such as 1000 pg/mL, 500 pg/mL, 250pg/mL, 125 pg/mL, 62.5 pg/mL, 31.25 pg/mL, 15.63 pg/mL, and the last EP tubes with Standard Diluent is the blank as 0 pg/mL. In order to guarantee the experimental results validity, please use the new standard solution for each experiment. 6. Streptavidin-HRP (1x) - Centrifuge the vial before opening. Streptavidin-HRP requires a 100-fold dilution. A suggested 100-fold dilution is 10 µL of Streptavidin-HRP with 990 µL of HRP Diluent. 7. TMB substrate - Aspirate the needed dosage of the solution with sterilized tips and do not dump the residual solution into the vial again.
Note	1. Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use. 2. Bacterialorfungal contamination of eithersamples or reagents or

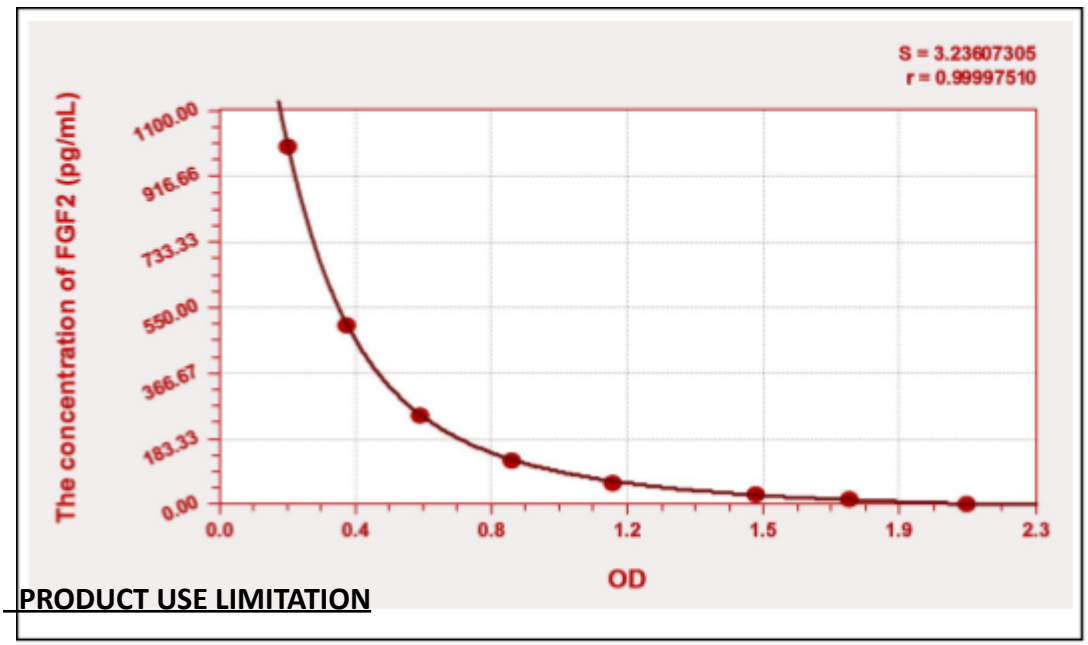
	cross-contamination between reagents may cause erroneous results. 3. All residual washing liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper directly into the wells. 4. If crystals have formed in the Wash Solution concentrate (25×), warm to room temperature and mix gently until the crystals are completely dissolved. 5. Prepare standards within15 minutes beforeassay. This standard can only be used once. 6. The TMB solution is light sensitive. Avoid prolonged exposure to light. Also, avoid contact of the TMB solution with metal to prevent color development. Warning TMB is toxic avoid direct contact with hands. Dispose of properly. If a dark blue color develops within a few minutes after preparation, this indicates that the TMB solution has been contaminated and must be discarded. 7. When pipetting reagents, maintain a consistent order of addition from well-to-well. This will ensure equal incubation times for all wells. Dispense the TMB solution within 15 minutes following the washing of the microtiter plate. 8. It is highly recommended to use the remaining reagents within 1 month provided this is prior to the expiration date of the kit. For the expiration date of the kit, please refer to the label on the kit box.
Samples preparation	1. Equilibrate all materials and prepared reagents to room temperature prior to use. Prior to use, mix all reagents thoroughly taking care not to create any foam within the vials. 2. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance. 3. Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
Assay Procedure	1. Bring all reagents and samples to room temperature before use. It is recommended that all samples,controls, and standards be assayed in duplicate. 2. Prepare all reagents, working standards, and samples as directed in the previous sections. 3. Refer to the Assay Layout Sheet to determine the number of wells to be used and put any remaining wells and the desiccant back into the pouch and seal the ziploc, store unused wells at 2 - 8°C. 4. Set a Blank well with Standard Diluent Buffer. Add 50 µL of Standard or Sample to per well. Add 50µL of Biotinylated -Conjugate(1x) to each well. Mix well, Cover with the adhesive films provided.Incubate for 1 hour at 37°C. 5. Aspirate each well and wash, repeating the process for a total of three washes. Wash by filling each well with Wash Buffer (200 µL) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. 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. 6. Add 100 µL of Streptavidin-HRP (1x) to each well. Cover with the adhesive films provided. Incubate for 1 hour at 37°C. 7. Aspirate each well and wash, repeating the process for a total of five washes. Wash by filling each well with Wash Buffer (200 µL) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. 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. 8. Add 90 µL of Substrate Solution to each well. Incubate for 20 minutes at 37°C. Keeping the plate away from drafts and other temperature fluctuations in the dark. Avoid placing the plate in direct light. 9. Add 50 µL of Stop Solution to each well. When the first four wells containing the highest concentration of standards develop obvious blue color. If color change does not appear uniform,gently tap the plate to ensure thorough mixing. 10. Determine the optical density of each well within 5 minutes, using a microplate reader set to 450nm. If wavelength correction is available, set to 540 nm or 570 nm. 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. 11. *Samples may require dilution. See Sample Preparation section.

Calculation of Results

This assay employs the competitive inhibition enzyme immunoassay technique, so there is an inverse correlation between Mouse FGF2 concentration in the sample and the assay signal intensity. Average the duplicate readings for each standard, control, and samples. Create a standard curve with the Mouse FGF2 concentration on the y-axis and absorbance on the x-axis. Draw the best fit straight line through the standard points and it can be determined by regression analysis. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor. Using some plot software, for instance, curve expert.

Concentration (pg/mL)	OD
1000	0.199
500	0.367
250	0.577
125	0.833
62.5	1.119
31.25	1.522
15.63	1.789
0	2.123



PRODUCT USE LIMITATION

Precision

Intra-assay Precision (Precision within an assay) : CV%<8%

Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays) : CV%<10%

Three samples of known concentration were tested in forty separate assays to assess inter-assay precision.

Recovery

Matrices listed below were spiked with certain level of recombinant FGF2 and the recovery rates were calculated by comparing the measured value to the expected amount of FGF2 in samples.

Matrix	Recovery range	Average
serum(n=5)	92-106%	99%
EDTA plasma(n=5)	90-105%	97%
heparin plasma(n=5)	78-90%	84%

Linearity

The linearity of the kit was assayed by testing samples spiked with appropriate concentration of FGF2 and their serial dilutions. The results were demonstrated by the percentage of calculated concentration to the expected.

Sample	1:2	1:4	1:8	1:16
serum(n=5)	86-93%	91-101%	87-98%	95-104%
EDTA plasma(n=5)	89-102%	92-101%	88-102%	87-102%
Heparin plasma(n=5)	83-98%	92-103%	86-97%	93-101%

Trouble shooting

Problem	Reason	Solution
Poorstandard curve	Inaccurate pipetting	Check pipettes
	Improper standard dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
	Incomplete washing and aspiration	Adequate washing and adequate aspiration
Low single	Inadequate reagent volumes added to wells	Calibrate pipettes and add adequate reagents
	The samples storage too long	Use new sample and repeat assay
	Incorrect incubation temperature	Ensure the incubator temperature is always 37°C
Poor Precision	Inaccurate pipetting	Check and calibrate pipettes
	Incomplete washing of wells	Ensure sufficient washing
	Contaminated wash buffer	Prepare fresh wash buffer
Poor repeatability	Time of addition is not consistent	Ensure that the sample time is consistent every time
	The washing conditions are not consistent	Ensure the frequency and strength of each wash are consistent