

Chicken FGF9(Fibroblast Growth Factor 9) ELISA Kit

Cat: ELK2937

For research use only. Not intended for diagnostic use.

Sensitivity: 30 pg/mL

Detection Range: 78.13-5000 pg/mL

Specificity: This assay has high sensitivity and excellent specificity for detection of Chicken FGF9.

No significant cross-reactivity or interference between Chicken FGF9 and analogues was observed.

Please refer to the outer packaging label of the kit for the specific shelf life.

KIT Components

Reagents	Quantity		Storage Condition
	48T	96T	
Pre-Coated Microplate	6 strips x 8 wells	12 strips x 8 wells	4°C/-20°C
Standard (Lyophilized)	1 vial	2 vials	4°C/-20°C
Biotinylated Antibody (100×)	60 µL	120 µL	4°C/-20°C
Streptavidin-HRP (100×)	60 µL	120 µL	4°C/-20°C
Standard/Sample Diluent Buffer	10 mL	20 mL	4°C/-20°C
Biotinylated Antibody Diluent	6 mL	12 mL	4°C/-20°C
HRP Diluent	6 mL	12 mL	4°C/-20°C
Wash Buffer (25×)	10 mL	20 mL	4°C/-20°C
TMB Substrate Solution	6 mL	10 mL	4°C/-20°C (store in dark)
Stop Reagent	3 mL	6 mL	4°C/-20°C
Plate Covers	1 piece	2 pieces	RT

Special Explanation

1. *If the kit is opened, Store the whole kit at 4°C. If the kit is not used up in 1 week. Store the Pre-Coated Microplate, Standard ,Biotinylated Antibody and Streptavidin-HRP at -20°C, the rest reagents at 4°C, please used up within 6 months.

*If the kit is not opened, store the whole kit: 4°C(short time storage, valid for 6 months); -20°C (long-term storage, valid for 1 year). Avoid repeated freeze-thaw cycles.

2. Do not use the kit beyond the expiration date.

3. If the whole kit is stored at -20°C, place the kit at 4°C the day before the experiment.

4. After opening the package, please check that all components are complete.

5. The cap must be tightened to prevent evaporation and microbial contamination. The reagents volume is slightly more than the volume marked on labels, please use accurate measuring equipment and do not pour directly into the vial.

All kit components have been formulated and quality control tested to function successfully. Do not mix or substitute reagents or materials from other kits, detection effect of the kit will not 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. Double distilled water or deionized water.
6. Single or multi-channel pipettes with high precision and disposable tips.
7. Precision pipettes to deliver 2 μ L to 1 mL volumes.

Safety Notes

1. This kit is only used for lab research and development and should not be used for human or animals.
2. Reagents should be regarded as hazardous substances and should be handled carefully and correctly.
3. Gloves, lab coats, and goggles should always be worn to avoid skin and eyes coming into contact

with Stop Solution and TMB. In case of contact, wash thoroughly with water.

Test Principle

The test principle applied in this kit is Sandwich enzyme immunoassay. The microtiter plate provided in this kit has been pre-coated with an antibody specific to Chicken FGF9. Standards or samples are added to the appropriate microtiter plate wells then with a biotin-conjugated antibody specific to Chicken FGF9. Next, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After TMB substrate solution is added, only those wells that contain Chicken FGF9, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of $450\text{nm} \pm 10\text{nm}$. The concentration of Chicken FGF9 in the samples is then determined by comparing the OD of the samples to the standard curve.

Sample Collection and Storage

Serum - Samples should be collected into a serum separator tube. After clotting for 2 hours at room temperature or overnight at 4°C, and then centrifuging at 1000 × g for 20 minutes. 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 at 1000 × g and 2-8°C for 15 minutes 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. Rinse the tissues in pre-cooled PBS to completely remove excess blood, and weigh them before homogenization.
2. Mince 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) (PBS can be used as the lysis buffer of most tissues) (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, too).
3. Ultrasound the obtained suspension with an ultrasonic cell disrupter until the solution is clear.
4. Then, centrifuge the homogenates for 5 minutes at 10000 × g and collect the supernatant and assay immediately or store in aliquots at ≤ -20°C.

***Note:** Tissue homogenates are recommended to be tested for protein concentration at the same time to obtain a more accurate concentration of the test substance per mg of protein. For protein detection, you can purchase our product: **BC016, BCA Protein concentration determination kit.**

Cell lysates - Cells need to be lysed before assaying according to the following directions.

1. Adherent cells should be washed by pre-cooled PBS gently, and then be detached with trypsin, and collect them by centrifugation at 1000 × g for 5 minutes (suspension cells can be collected by centrifugation directly).
2. Wash cells 3 times in pre-cooled PBS.
3. Then, resuspend the cells in fresh lysis buffer with concentration of 10⁷ cells/mL. If it is necessary, the cells could be subjected to ultrasonication until the solution is clear.
4. Centrifuge at 1500 × g for 10 minutes at 2-8°C to remove cellular debris. Assay immediately or store in aliquots at ≤ -20°C.

Urine - Collect the first urine of the day (mid-stream) and discharge it directly into a sterile container. Centrifuge to remove particulate matter, assay immediately or aliquot and store at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

Saliva - Collect saliva using a collection device or equivalent. Centrifuge samples at $1000 \times g$ at $2-8^{\circ}\text{C}$ for 15 minutes. Remove particulates and assay immediately or store samples in aliquot at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

Feces - Dry feces were collected as much as possible, weighing more than 50 mg. The feces were washed three times with PBS (w:v = 1:9, e.g. 900 μL lysis buffer is added in 100 mg feces), sonicated (or mashed) and centrifuged at $5000 \times g$ for 10 minutes, where the supernatant was collected for testing.

Cell culture supernatants and other biological fluids - Centrifuge samples at $1000 \times g$ for 20 minutes. 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.

Cerebrospinal fluid (CSF) - Remove particulates by centrifugation and assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

Notes

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 it 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 dilutions according to the actual situation (it is recommended to do preliminary experiment to determine the dilution ratio).

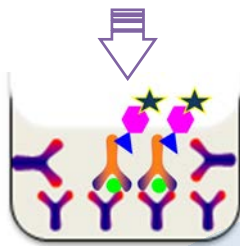
Summary



1. After the kit is equilibrated at room temperature, add 100 μL of Standard Working Buffer (gradually diluted according to the instructions) or 100 μL of sample to each well, and incubate at 37°C for 80 minutes.



2. Discard the liquid in the plate, add 200 μL 1 \times Wash Buffer to each well, and wash the plate 3 times. After pat it dry against clean absorbent paper, add 100 μL Biotinylated Antibody Working Solution (1 \times) to each well, incubate at 37°C for 50 minutes.



3. Discard the liquid in the plate, add 200 μL 1 \times Wash Buffer to each well, and wash the plate 3 times. After pat it dry against clean absorbent paper, add 100 μL 1 \times Streptavidin-HRP Working Solution to each well, incubate at 37°C for 50 minutes.



4. Discard the liquid in the plate, add 200 μL 1 \times Wash Buffer to each well, and wash the plate 5 times. After pat it dry against clean absorbent paper, add 90 μL TMB Substrate Solution to each well, incubate at 37°C for 20 minutes in the dark.



5. Add 50 μL Stop Solution to each well, shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm immediately, calculation of the results.

Reagent Preparation

1. Bring all kit components and samples to room temperature (18-25°C) before use. Make sure all components are dissolved and mixed well before using the kit.
2. If the kit will not be used up in 1 time, please only take out strips and reagents for present experiment, and save the remaining strips and reagents as specified.
3. Dilute the 25× Wash Buffer into 1× Wash Buffer with double-distilled Water.
4. **Standard Working Solution** - Centrifuge the Standard at 1000 × g for 1 minute. Reconstitute the Standard with 1.0 mL of Standard Diluent Buffer, kept for 10 minutes at room temperature, shake gently (not to foam). The concentration of the Standard in the stock solution is 5000 pg/mL. Please prepare 7 tubes containing 0.5 mL Standard Diluent Buffer and use the Diluted Standard to produce a double dilution series according to the picture shown below. To mix each tube thoroughly before the next transfer, pipette the solution up and down several times. Set up 7 points of Diluted Standard such as 5000 pg/mL, 2500 pg/mL, 1250 pg/mL, 625 pg/mL, 312.5 pg/mL, 156.25 pg/mL, 78.13 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. When diluting the Standard from high concentration to low concentration, replace the pipette tip for each dilution. Note: the last tube is regarded as a **Blank** and **do not** pipette solution into it from the former tube.



5. **1× Biotinylated Antibody and 1× Streptavidin-HRP** - Briefly spin or centrifuge the stock Biotinylated Antibody and Streptavidin-HRP before use. Dilute them to the working concentration 100-fold with Biotinylated Antibody Diluent and HRP Diluent, respectively.
6. **TMB Substrate Solution** - Aspirate the needed dosage of the solution with sterilized tips and **do not**

dump the residual solution into the vial again.

Notes

1. After receive the kit, please store the reagents according to the instructions. The plates can be disassembled to single strips. Please use it in batches on demands.
2. The test tubes, pipette tips and reagents used in the experiment are all disposable and are strictly prohibited from being reused; otherwise the experiment results will be affected. Kit reagents of different batches cannot be mixed (except TMB, Washing Buffer and Stop Reagent).
3. Lyophilized Standards, Biotinylated Antibody, and Streptavidin-HRP are small in volume and may be scattered in various parts of the tube during transportation. Please centrifuge at $1000 \times g$ for 1 minute before use. Then, carefully pipette 4-5 times to mix the Solution. Please configure the Standard, Biotinylated Antibody and Streptavidin-HRP Working Solution according to the required amount, and use the corresponding Dilution Solution, cannot be mixed used.
4. Bring all reagents to room temperature ($18-25^{\circ}\text{C}$) before use. If crystals form in the concentrate ($25\times$), it is a normal phenomenon. Heat it to room temperature (the heating temperature should not exceed 40°C), gently Mix until crystals are completely dissolved.
5. Prepare to dissolve Standard within 15 minutes before the test. This Standard Working Solution can only be used once. If the dissolved Standard is not used up, please discard it. The sample addition needs to be rapid. Each sample addition should preferably be controlled within 10 minutes. To ensure experimental accuracy, it is recommended to test duplicate wells, and when pipetting reagents, keep a consistent order of additions from 1 well to another, this will ensure the same incubation time for all wells.
6. During the washing process, the residual washing liquid in the reaction well should be patted dry on absorbent paper. Do not put the paper directly into the reaction well to absorb water. Before reading, pay attention to remove the residual liquid and fingerprints at the bottom, so as not to affect the microplate reader reading.
7. TMB Substrate Solution is light-sensitive, avoid prolonged exposure to light. **Dispense the TMB Substrate Solution within 15 minutes following the washing of the microtiter plate.** In addition, avoid contact between TMB Substrate Solution and metal to prevent color development. TMB is contaminated if it turns blue color before use and should be discarded. TMB is toxic, avoid direct

5. Add 100 μ L of Streptavidin-HRP Working Solution to each well, cover the wells with the plate sealer and incubate for 50 minutes at 37°C.
6. Repeat the aspiration, wash process for total 5 times as conducted in step 2.
7. Add 90 μ L of TMB Substrate Solution to each well. Cover with a new Plate Cover. Incubate for 20 minutes at 37°C (Don't exceed 30 minutes) in the dark. The liquid will turn blue by the addition of TMB Substrate Solution. Preheat the Microplate Reader for about 15 minutes before OD measurement.
8. Add 50 μ L of Stop Reagent to each well. The liquid will turn yellow by the addition of Stop Reagent. Mix the liquid by tapping the side of the plate. If color change does not appear uniform, gently tap the plate to ensure thorough mixing. The insertion order of the Stop Reagent should be the same as that of the TMB Substrate Solution.
9. Wipe off any drop of water and fingerprint on the bottom of the plate and confirm there is no bubble on the surface of the liquid. Then, run the microplate reader and conduct measurement at 450 nm immediately.

Description of results	Possible reason	Recommendations and precautions
	The liquid filling head of the plate washer is blocked, resulting in unsatisfactory liquid addition or large residual amount of liquid suction, resulting in the color of plate is chaotic and irregular	Unblock the liquid addition head, so that each well is filled with washing liquid when washing the plate and the residual amount should be small when aspirating liquid.
The color of plate is chaotic and irregular	Incomplete centrifugation of the sample, resulting in coagulation in the reaction well or interference of sediment or residual cellular components	Serum plasma should be fully centrifuged at 3000 rpm for more than 6 minutes
	The sample is stored for too long time, resulting in contamination.	Samples should be kept fresh or stored at low temperature to prevent contamination
	Incorrect preparation of Washing Solution or direct misuse of concentrated Washing Solution	Please configure according to the manual