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Product Data Sheet

IK4128 Human FAS ELISA Kit

Size: 96 T

Range: 62.5 pg/ml – 4000pg/ml

Sensitivity: < 15 pg/ml

Specificity: No detectable cross-reactivity with any other cytokine.

Applications: For quantitative detection of human FGF9 in sera, plasma, body fluids, tissue lysates or cell culture supernates.

Principle: Immunological Science's human FGF9 ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. Human FGF9 specific-specific monoclonal antibodies (clone No. 36912) were precoated onto 96-well plates. The human specific detection polyclonal antibodies were biotinylated. The test samples and biotinylated detection antibodies were added to the wells subsequently and then followed by washing with PBS or TBS buffer. Avidin-Biotin-Peroxidase Complex was added and unbound conjugates were washed away with PBS or TBS buffer. HRP substrate TMB was used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the human FGF9 amount of sample captured in plate.

Background: Fibroblast growth factor-9 (FGF-9) is a steroid-regulated mitogen and survival factor for nerve and mesenchymal cells.The human FGF-9 cDNA cloned by using oligonucleotide probes encodes a polypeptide consisting of 208 amino acids. Sequence similarity to other members of the FGF family has been estimated to be around 30%. FGF-9 is an autocrine estromedin endometrial stromal growth factor that plays roles in cyclic proliferation of uterine endometrial stroma. FGF9 is produced and secreted by the prostatic stromal cells. It is a potent mitogen for both prostatic epithelial and stromal cells in culture. FGF9 is an abundant secreted growth factor that can act as both a paracrine mitogen for epithelial cells and an autocrine mitogen for stromal cells. Overexpression of this paracrine and autocrine growth factor may play an important role in the epithelial and stromal proliferation in benign prostatic hyperplasia.The standard product used in this kit is recombinant human FGF9, consisting of 208 amino acids with the molecular mass of 23KDa. As a result of glycosylation, the molecular mass is 25-27KDa.

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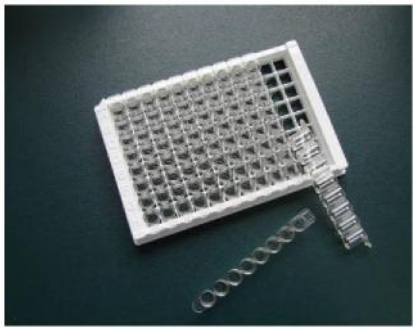
KIT COMPONENTS



1. Lyophilized recombinant human FGF9 standard: 10ng/tube×2.
2. One 96-well plate precoated with anti- human FGF9 antibody.
3. Sample diluent buffer: 30 ml
4. Biotinylated anti- human FGF9 antibody: 130µl, dilution 1:100.
5. Antibody diluent buffer: 12ml.
6. Avidin-Biotin-Peroxidase Complex (ABC): 130µl, dilution 1:100.
7. ABC diluent buffer: 12ml.
8. TMB color developing agent: 10ml.
9. TMB stop solution: 10ml.

Material Required But Not Provided

1. Microplate reader in standard size.
2. Automated plate washer.
3. Adjustable pipettes and pipette tips. Multichannel pipettes are recommended in the condition of large amount of samples in the detection.
4. Clean tubes and Eppendorf tubes.
5. Washing buffer (neutral PBS or TBS).
    - Preparation of 0.01M TBS: Add 1.2g Tris, 8.5g Nacl; 450µl of purified acetic acid or 700µl of concentrated hydrochloric acid to 1000ml H2 O and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.
    - Preparation of 0.01 M PBS: Add 8.5g sodium chloride, 1.4g Na2 HPO4 and 0.2g NaH2 PO4 to 1000ml distilled water and adjust pH to 7.2-7.6.
    - Finally, adjust the total volume to 1L.

Notice for Application of Kit

1. Before using Kit, spin tubes and bring down all components to bottom of tube.
2. Duplicate well assay was recommended for both standard and sample testing.
3. Don't let 96-well plate dry, dry plate will inactivate active components on plate.
4. In order to avoid marginal effect of plate incubation due to temperature difference (reaction may be stronger in the marginal wells), it is suggested that the diluted ABC and TMB solution will be pre-warmed in 37℃for 30 min before using.

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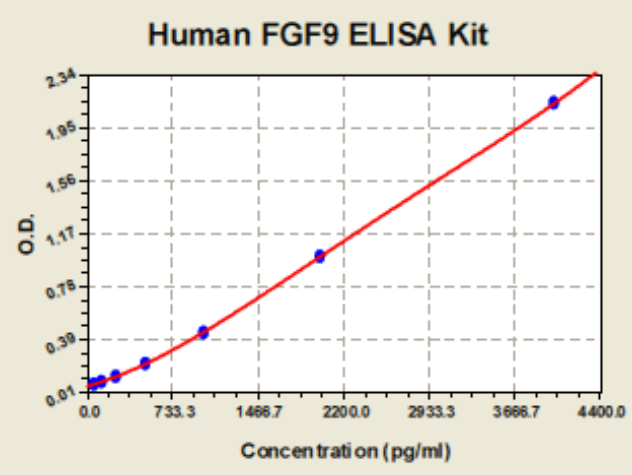
Human FGF9 ELISA Kit-1X96 Well Plate Image



Storage : Store at 4℃for frequent use, at -20℃for infrequent use. Avoid multiple freeze-thaw cycles (Shipped with wet ice.)

Expiration: Four months at 4℃and eight months at -20℃

For Research use only; not for use as a diagnostic

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Human FGF9 ELISA Kit

Preparation

- **Plate Washing**

  Discard the solution in the plate without touching the side walls. Blot the plate onto paper towels or other absorbent material. Soak each well with at least 0.3 ml PBS or TBS buffer for 1~2 minutes. Repeat this process two additional times for a total of THREE washes.

  Note: For automated washing, aspirate all wells and wash THREE times with PBS or TBS buffer, overfilling wells with PBS or TBS buffer. Blot the plate onto paper towels or other absorbent material.

- **Sample Preparation and Storage**

  Store samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freeze samples at -70°C. Avoid repeated freeze-thaw cycles.

  - **Cell culture supernate, tissue lysate or body fluids**: Remove particulates by centrifugation, analyze immediately or aliquot and store at -20°C
  - **Serum**: Allow the serum to clot in a serum separator tube (about 30 min) at room temperature. Centrifuge at approximately 1000 X g for 15 min. Analyze the serum immediately or aliquot and store frozen at -70°C.
  - **Plasma**: Collect plasma using EDTA as an anticoagulant. Centrifuge for 15 min at 1000 x g within 30 min of collection. Analyze immediately or aliquot and store frozen at -70°C. Heparin and citrate are not recommended as the anticoagulant.

- **Sample Dilution Guideline**

  The user needs to estimate the concentration of the target protein in the sample and select a proper dilution factor so that the diluted target protein concentration falls near the middle of the linear regime in the standard curve. Dilute the sample using the provided diluent buffer. The following is a guideline for sample dilution. Several trials may be necessary in practice. **The sample must be well mixed with the diluents buffer.**

  - **High target protein concentration (40-400ng/ml).** The working dilution is 1:100. i.e. Add 1 µl sample into 99 µl sample diluent buffer.
  - **Medium target protein concentration (4-40ng/ml).** The working dilution is 1:10. i.e. Add 10 µl sample into 90 µl sample diluent buffer.
  - **Low target protein concentration (62.5-4000pg/ml).** The working dilution is 1:2. i.e. Add 50 µl sample to 50 µl sample diluent buffer.
  - **Very Low target protein concentration (≤62.5pg/ml).** No dilution necessary, or the working dilution is 1:2.

- **Reagent Preparation and Storage**

  A. Reconstitution of the human FGF9 standard: FGF9 standard solution should be prepared no more than 2 hours prior to the experiment. Two tubes of FGF9 standard (10ng per tube) are included in each kit. Use one tube for each experiment.

     a. 10,000pg/ml of human FGF9 standard solution: Add 1 ml sample diluent buffer into one tube, keep the tube at room temperature for 10 min and mix thoroughly.

     b. 4000pg/ml of human FGF9 standard solution: Add 0.4 ml of the above 10ng/ml FGF9 standard solution into 0.6 ml sample diluent buffer and mix thoroughly.

c. 2000pg/ml→62.5pg/ml of human FGF9 standard solutions: Label 6 Eppendorf tubes with 2000pg/ml, 1000pg/ml, 500pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml, respectively. Aliquot 0.3 ml of the sample diluent buffer into each tube. Add 0.3 ml of the above 4000pg/ml FGF9 standard solution into 1st tube and mix. Transfer 0.3 ml from 1st tube to 2nd tube and mix. Transfer 0.3 ml from 2nd tube to 3rd tube and mix, and so on.

Note: The standard solutions are best used within 2 hours. The 10 ng/ml standard solution may be stored at 4°C for up to 12 hours, or at -20°C for up to 48 hours. Avoid repeated freeze-thaw cycles.

B. Preparation of biotinylated anti-human FGF9 antibody working solution: The solution should be prepared no more than 2 hours prior to the experiment.
   a. The total volume should be: 0.1ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)
   b. Biotinylated anti-human FGF9 antibody should be diluted in 1:99 with the antibody diluent buffer and mixed thoroughly.

C. Preparation of Avidin-Biotin-Peroxidase Complex (ABC) working solution: The solution should be prepared no more than 1 hour prior to the experiment.
   a. The total volume should be: 0.1ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)
   b. Avidin- Biotin-Peroxidase Complex (ABC) should be diluted in 1:99 with the ABC dilution buffer and mixed thoroughly.

Assay Procedure

The ABC working solution and TMB color developing agent must be kept warm at 37°C for 30 min before use. When diluting samples and reagents, they must be mixed completely and evenly. Standard FGF9 detection curve should be prepared for each experiment. The user will decide sample dilution fold by crude estimation of FGF9 amount in samples.

1. Aliquot 0.1ml per well of the 4000pg/ml, 2000pg/ml, 1000pg/ml, 500pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml human FGF9 standard solutions into the precoated 96-well plate. Add 0.1ml of the sample diluent buffer into the control well (Zero well). Add 0.1ml of each properly diluted sample of human sera, plasma, body fluids, tissue lysates or cell culture supernatants to each empty well. **See "Sample Dilution Guideline" above for details.** We recommend that each human FGF9 standard solution and each sample is measured in duplicate.
2. Seal the plate with the cover and incubate at 37°C for 90 min.
3. Remove the cover, discard plate content, and blot the plate onto paper towels or other absorbent material. Do NOT let the wells completely dry at any time.
4. Add 0.1ml of biotinylated anti-human FGF9 antibody working solution into each well and incubate the plate at 37°C for 60 min.
5. Wash the plate three times with 0.01M TBS or 0.01M PBS, and each time let washing buffer stay in the wells for 1 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material.
6. Add 0.1ml of prepared ABC working solution into each well and incubate the plate at 37°C for 30 min.
7. Wash plate 5 times with 0.01M TBS or 0.01M PBS, and each time let washing buffer stay in the wells for 1-2 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material.
8. Add 90 µl of prepared TMB color developing agent into each well and incubate plate at 37°C for 25-30 min (shades of blue can be seen in the wells with the four most concentrated human FGF9 standard

solutions; the other wells show no obvious color).

9. Add 0.1ml of prepared TMB stop solution into each well. The color changes into yellow immediately.
10. Read the O.D. absorbance at 450nm in a microplate reader within 30 min after adding the stop solution.

For calculation, (the relative $O.D._{450}$) = (the $O.D._{450}$ of each well) – (the $O.D._{450}$ of Zero well). The standard curve can be plotted as the relative $O.D._{450}$ of each standard solution (Y) vs. the respective concentration of the standard solution (X). The human FGF9 concentration of the samples can be interpolated from the standard curve. **Note:** if the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

Summary

1. Add samples and standards and incubate the plate at 37°C for 90 min. Do not wash.
2. Add biotinylated antibodies and incubate the plate at 37°C for 60 min. Wash plate 3 times with 0.01M TBS.
3. Add ABC working solution and incubate the plate at 37°C for 30 min. Wash plate 5 times with 0.01M TBS.
4. Add TMB color developing agent and incubate the plate at 37°C for 25-30 min.
5. Add TMB stop solution and read.

Typical Data Obtained from Human FGF9

(TMB reaction incubate at 37°C for 30 min)

Concen-tration	0.0pg/ml	62.5pg/ml	125pg/ml	250pg/ml	500pg/ml	1000pg/ml	2000pg/ml	4000pg/ml
O.D	0.051	0.071	0.082	0.122	0.217	0.450	1.006	2.130

Typical Human FGF9 ELISA Kit Standard Curve

This standard curve was generated for demonstration purpose only. A standard curve must be run with each assay.