IMMUNOLOGICAL SCIENCES

Product Data Sheet

IK4123 Human EGFR ELISA Kit

Size: 96 T

Range: 62.5 pg/ml - 4000pg/ml

Sensitivity: < 1 pg/ml

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

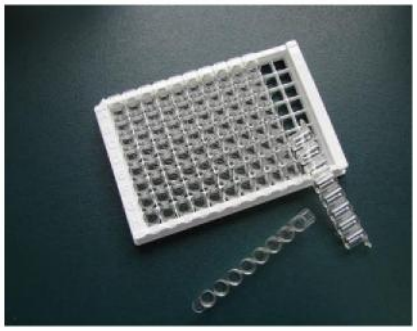
Applications: For quantitative detection of human EGFR in sera, plasma, body fluids, tissue lysates or cell culture supernates.

Principle: Immunological Science's human EGFR ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. Human EGFR specific-specific polyclonal antibodies 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 EGFR amount of sample captured in plate.

Background: The epidermal growth factor receptor (EGFR; ErbB-1; HER1 in humans) is the cell-surface receptor for members of the epidermal growth factor family (EGF-family) of extracellular protein ligands. It is a member of the ErbB family of receptors, a subfamily of four closely related receptor tyrosine kinases: EGFR (ErbB-1), HER2/c-neu (ErbB-2), Her 3 (ErbB-3) and Her 4 (ErbB-4). EGFR exists on the cell surface and is activated by binding of its specific ligands, including epidermal growth factor and transforming growth factor α(TGFα). EGFR and its ligands are cell signaling molecules involved in diverse cellular functions, including cell proliferation, differentiation, motility, and survival, and in tissue development. Mutations that lead to EGFR overexpression (known as upregulation) or overactivity have been associated with a number of cancers, including lung cancer and glioblastoma multiforme. In this latter case a more or less specific mutation of EGFR, called EGFRvIII is often observed.

Product Data Sheet

KIT COMPONENTS



1. Lyophilized recombinant human EGFR standard: 10ng/tube×2.
2. One 96-well plate precoated with anti- human EGFR antibody.
3. Sample diluent buffer: 30 ml
4. Biotinylated anti- human EGFR 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.

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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Web-site: www.immunologicalsciences.com
E-Mail: info@immunologicalsciences.com

Protocol for Elisa Kit
Human EGFR 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 -20℃. 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 2 hours) at room temperature. Centrifuge at approximately 1000 X g for 10 min. Analyze the serum immediately or aliquot and store frozen at -20°C.
  - **Plasma**: Collect plasma using EDTA as an anticoagulant. Centrifuge for 10 min at 1000 x g within 30 min of collection. Analyze immediately or aliquot and store frozen at -20°C.

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

     a. 10,000pg/ml of human EGFR 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 EGFR standard solution: Add 0.4 ml of the above 10ng/ml EGFR standard solution into 0.6 ml sample diluent buffer and mix thoroughly.

     c. 2000pg/ml→62.5pg/ml of human EGFR 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 EGFR 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 EGFR 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 EGFR 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 EGFR detection curve should be prepared for each experiment. The user will decide sample dilution fold by crude estimation of EGFR 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 EGFR 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 EGFR 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 EGFR 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 15-19 min (shades of blue can be seen in the wells with the four most concentrated human EGFR 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 EGFR 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 15-19 min.
5. Add TMB stop solution and read.

Typical Data Obtained from Human EGFR

(TMB reaction incubate at 37°C for 17 min)

Concen-tration	0.0pg/ml	62.5pg/ml	125pg/ml	250pg/ml	500pg/ml	1000pg/ml	2000pg/ml	4000pg/ml
O.D	0.013	0.164	0.254	0.485	0.858	1.361	1.871	2.299

Typical Human EGFR ELISA Kit Standard Curve

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