



AEK0006

## Human Fas/TNFRSF6 ELISA Kit

Human Fas/Tumor Necrosis Factor Receptor Superfamily Member 6 (Fas/TNFRSF6) is an ELISA Kit designed for the quantitative measurement of serum, plasma and cell culture supernatant in the human basic fibroblast growth factor (FGF basic) concentration.

**This product is only used for scientific research. It is not a diagnostic reagent and cannot be used for clinical diagnosis.**

### 1. Product introduction

### 1. Background introduction

Fas, also known as APO-1, CD95 or TNFRSF6, is a cell surface receptor that can transduce apoptosis signals. It is one of two apoptosis pathways, and the other is the mitochondrial pathway. Once bound to the ligand, Fas forms a death-inducing signal complex. Membrane anchoring of Fas ligand trimers on the surface of adjacent cells leads to Fas oligomerization. Recent mouse model studies have shown that Fas promotes tumor growth, but the human cancer genomics database shows that Fas can act as a tumor suppressor. In AOM-DSS-induced colon cancer and MCA-induced sarcoma mouse models, the results showed that Fas is a tumor suppressor. In addition, Fas can also mediate the anti-tumor cytotoxicity of tumor-specific cytotoxic T cells.

Studies have reported that serum Fas levels in patients with cancer and autoimmune diseases are elevated, the former may be derived from the tumor cells themselves.

### 2. Detection principle

This kit uses double antibody sandwich enzyme-linked immunosorbent detection technology. The specific anti-human Fas antibody is pre-coated on a high-affinity ELISA plate. Standards, samples to be tested and biotinylated detection antibody are added to the wells of the enzyme-labeled plate. After incubation, the Fas present in the sample binds to the solid-phase antibody and the detection antibody. After washing to remove unbound substances, horseradish peroxidase-labeled streptavidin (Streptavidin-HRP) was added. After washing, add TMB, a chromogenic substrate, to avoid light for color development. The intensity of the color response is directly proportional to the concentration of Fas in the sample. Add stop solution to stop the reaction, and measure the absorbance value at 450 nm wavelength (reference wavelength 570-630 nm).

### 3. Limitations of kit detection

1) Please use within the validity period marked on this kit.  
2) The reagents of the kit cannot be mixed with reagents of other batch numbers or reagents from other sources.  
3) Any changes in standard dilution, operators, pipetting techniques, washing techniques, incubation temperature, and kit storage time will affect the binding reaction.

4) This kit is designed to remove or reduce some endogenous interference factors in biological samples, and not all possible influencing factors have been removed.

### 2. Basic information

#### 1. Materials provided by the kit

Component number EK1F01-24 EK1F01-48 EK1F01-96

Pre-coated ELISA plate EK1F01P 24T 48T 96T

Standard EK1F01S 1 vial 1 vial 2 vials

Detection antibody EK1F01D 1 vial 1 vial 1 vial

Horseradish peroxidase

Tagged streptavidin E0290 1 vial 1 vial 1 vial

10× Detection Buffer E0310 5 ml 5 ml 5 ml

Chromogenic substrate TMB E0230 3 ml 6 ml 11 ml

Stop solution E0300 11 ml 11 ml 11 ml

20× Solution E0281 50 ml 50 ml 50 ml

Sealing film E0200 3 6 6

2. Materials and equipment not provided

1) A microplate reader capable of detecting absorbance at 450 nm, with a reference wavelength of 570 nm or 630 nm

2) Pipette, pipette tip, and sample tank

3) Prepare test tubes, centrifuge tubes, graduated cylinders, etc. for reagents

4) Distilled water or deionized water

5) Vortex oscillator, microplate oscillator

3. Storage

The kit is stored at 2-8° C, and the expiration date is indicated on the label. Only properly stored reagents are guaranteed. If the components of the kit need to be used again, make sure that they have not been contaminated since the last use.

Store the unopened kit at 2-8° C.

Please use it within the validity period.

beat

Open

of

try

Agent

Box

Or

Heavy

Group

try

Agent 1 x lotion

1 × detection buffer

Stop solution

Chromogenic substrate TMB

Detection antibody

Horseradish peroxidase-labeled streptavidin at 2-8°C,

It can be stored for about 1 month.

The standard product can be stored for about 1 month at -20° C.

Discard after use.

Please put the unused slats back into the aluminum foil bag and seal the seal. It can be stored for about 1 month at 2-8° C.

#### 4. Matters needing attention

1) All chemical reagents should be considered as potentially hazardous.

2) It is recommended that only staff with good laboratory training can operate this kit. Please wear suitable protective equipment during operation, such as white

coat, latex gloves, safety glasses, etc.

3) Please avoid reagent contact with skin and eyes. In case of accidental contact, please wash immediately with plenty of water.

4) The stop solution in the kit is an acidic solution. When using the stop solution, please wear protective clothing and facilities to protect your eyes, hands and face.

5) This kit is used for scientific research and cannot be used for diagnosis and treatment.

6) Please do not use other lot numbers or reagents from other sources to replace the reagents in this kit.

7) Please do not use expired reagents.

8) Please avoid strong light during the storage or incubation of the kit.

9) Please do not eat or drink in the area where the kit is operated or the sample is processed.

10) Do not allow reagents or samples to contact the skin and mucous membranes.

11) Please wear latex or disposable gloves when handling the kit or handling samples.

- 12) Avoid contact with oxidizing reagents and metals from the chromogenic substrate.
- 13) Avoid the generation of aerosols.
- 14) In order to avoid microbial contamination and cross-contamination between reagents and samples, please use disposable tips.
- 15) Use clean containers to prepare reagents.
- 16) Exposure to an acidic environment will inhibit binding.
- 17) The preparation of reagents must use distilled water or deionized water.
- 18) The chromogenic substrate must be equilibrated to room temperature before use.
- 19) The samples may contain infectious pathogens. The preferred method for handling samples and possible contaminated materials is 121.5° C for at least 1 hour.
- 20) Disposal of liquid waste. For liquid waste without acid, add 1.0% sodium hypochlorite and soak for 30 minutes. Please neutralize the acid-containing liquid

waste before adding sodium hypochlorite.

#### 5. Technical points

- 1) When re-dissolving or mixing protein, always avoid air bubbles.
- 2) To avoid cross-contamination, please replace the pipette tip when adding standard samples, sample samples, and different reagents. Different reagents use different loading tanks.
- 3) When using an automatic plate washer, after adding the washing solution, set a 30-second soaking program, or turn the microplate 180 degrees in different washing steps, which can improve the accuracy of the analysis.
- 4) To ensure the accuracy of the results, seal the plate with the sealing film during incubation.
- 5) The chromogenic substrate should be colorless before adding. Keep the chromogenic substrate always in a light-proof state.
- 6) The order of addition of the stop solution should be the same as the order of addition of the chromogenic substrate.
- 7) After adding the stop solution, the color of the substrate should change from blue to yellow. If the substrate is green, it means that the stop solution and the color-developing substrate are not sufficiently mixed.
- 8) It is recommended that all test samples and standard products have multiple holes in the test.
- 9) In any case, avoid touching the inner surface of the microplate.

#### Three, detection steps

##### 1. Sample collection and storage

Cell culture supernatant

Centrifuge at  $300 \times g$  for 10 minutes to remove the sediment, and then detect it immediately, or in aliquots, and store below  $-20^{\circ} C$ .

#### Serum sample

Collect serum in centrifuge tube. After 30 minutes of agglutination, the blood sample was centrifuged at  $1,000 \times g$  for 10 minutes. Test immediately after drawing the serum sample, or aliquot and store it below  $-20^{\circ} C$ .

#### Plasma sample

EDTA, sodium citrate or heparin anticoagulation to collect plasma samples. Collect samples by centrifugation at  $1,000 \times g$  for 30 minutes. Immediately detect, or sub-package, and store below  $-20^{\circ} C$ .

This kit may be suitable for other biological samples. Cell culture supernatant, serum and plasma have been validated.

Note: Before testing, the visible precipitation in the sample must be removed. Do not use samples with severe hemolysis or hyperlipidemia. The samples should be aliquoted and stored at  $-20^{\circ} C$  to avoid loss of human Fas activity. If tested within 24 hours. The samples can be stored at  $2-8^{\circ} C$ .

Avoid repeated freezing and thawing of samples. Before testing, the frozen sample should be slowly returned to room temperature and mixed gently.

#### 2. Sample preparation

Normal serum plasma samples need to be diluted 20 times. Recommend  $20 \mu l$  sample +  $380 \mu l$   $1 \times$  detection buffer.

#### 3. Reagent preparation

Please return all reagents and samples to room temperature before testing.

If the concentrated reagents crystallize, warm the bath at  $37^{\circ} C$  until all the crystals are dissolved.

#### $1 \times$ Lotion

Draw  $20 \times$  concentrated lotion from 50 ml to 1 L measuring cylinder, add distilled water to 1,000 ml, and mix gently to avoid foam. Transfer to a clean bottle. Stored at  $2-25^{\circ} C$ ,  $1 \times$  lotion can be stored stably for 30 days.

#### $1 \times$ detection buffer

Pipette  $10 \times$  concentrated detection buffer from 5 ml to 100 ml graduated cylinder, add distilled water to 50 ml, and mix gently to avoid foam. Stored at  $2-8^{\circ} C$ ,  $1 \times$  detection buffer can be stored stably for 30 days.

#### Detection antibody

Mix well before dilution. Dilute the concentrated detection antibody 1:100 with  $1 \times$  detection buffer according to the number of standards and samples to be tested.

Note: Please use the diluted detection antibody within 30 minutes.

Horseradish peroxidase labeled streptavidin

Mix well before dilution. Dilute the concentrated horseradish peroxidase-labeled streptavidin at a ratio of 1:100 with 1× detection buffer according to the number of standards and samples to be tested.

Note: Please use the diluted horseradish peroxidase-labeled streptavidin within 30 minutes.

Sample dilution

If the sample needs to be diluted, please dilute the serum/plasma sample with the 1× detection buffer provided in the kit, and dilute the cell culture supernatant with cell culture medium.

Human Fas Standard

Centrifuge briefly before opening the lid, and re-dissolve the human Fas standard with distilled water. The re-dissolved volume is marked on the label of the human Fas standard. Vortex gently to ensure thorough mixing. The concentration of the standard after reconstitution is 4,000 pg/ml. After re-dissolving, let it stand for 10-30 minutes. Mix well before dilution.

Please use polypropylene tubing for standard dilution.

Preparation of the standard curve of serum/plasma samples:

Take 230 μl of concentrated human Fas standard and add 230 μl 1× detection buffer as the highest concentration of the standard curve (2,000 pg/ml). Add 230 μl 1× detection buffer to each test tube. Use high-concentration standards to make 1:1 serial dilutions. Every time you pipette, make sure to mix well. Take 1× detection buffer as the zero concentration of the standard curve.

Preparation of the standard curve of cell culture supernatant samples:

Take 230 μl of concentrated human Fas standard and add 230 μl cell culture medium as the highest concentration of the standard curve (2,000 pg/ml). Add 230 μl of cell culture medium to each test tube. Use high-concentration standards to make 1:1 serial dilutions. Make sure to mix well each time you pipette. Take the cell culture medium as the zero concentration of the standard curve.

#### 4. Detection steps

Please equilibrate all reagents and samples to room temperature before testing.

- 1) Prepare all required reagents and working concentration standards.
- 2) Remove the unnecessary slats, put them back in the aluminum foil bag with desiccant, and re-seal the seal.
- 3) Soak the ELISA plate: Add 300 μl 1× lotion and let it stand and soak for 30 seconds. In order to obtain the desired experimental results, soaking is necessary.

After discarding the lotion, pat the microplate dry on absorbent paper. After washing the plate, please use the microplate immediately and do not let the microplate dry.

4) Add standard product: add 100  $\mu$ l standard product diluted 2 times to the standard product well. Add 100  $\mu$ l  $1 \times$  detection buffer (serum/plasma sample) or medium (cell culture supernatant sample) to the blank well.

5) Add sample: serum/plasma: add 100  $\mu$ l of pre-diluted sample to the sample well. Cell culture supernatant: add 100  $\mu$ l of cell culture supernatant to the sample well. (For sample dilution, please refer to "Sample Preparation" on page 6).

6) Add detection antibody: add 50  $\mu$ l diluted detection antibody (1:100 dilution) to each well. Ensure that steps 4, 5, and 6 continue to add samples without interruption. The sample addition process is completed within 15 minutes.

7) Incubation: Use sealing film to seal the plate. Shake at 300 rpm and incubate at room temperature for 2 hours.

8) Washing: Discard the liquid, add 300  $\mu$ l washing solution to each well to wash the plate, and wash 6 times. Each time you wash the plates, pat them dry on absorbent paper. In order to obtain the ideal experimental performance, the residual liquid must be completely removed.

9) Incubation with enzyme: Add 100  $\mu$ l of diluted horseradish peroxidase-labeled streptavidin (1:100 dilution) to each well.

10) Incubation: Use a new sealing film to seal the plate. Shake at 300 rpm and incubate at room temperature for 45 minutes.

11) Washing: Repeat step 8.

12) Add substrate for color development: add 100  $\mu$ l of color substrate TMB to each well, protect from light, and incubate at room temperature for 5-30 minutes.

13) Add stop solution: add 100  $\mu$ l stop solution to each well. The color changed from blue to yellow. If the color is green or the color change is obviously uneven, tap the board frame lightly to mix well.

14) Detection reading: within 30 minutes, use a microplate reader for dual-wavelength detection, and determine the OD value at 450 nm maximum absorption wavelength and 570 nm or 630 nm reference wavelength. The OD value after calibration is the measured value of 450 nm minus the measured value of 570 nm or 630 nm. Using only 450 nm measurement will result in a high OD value and a decrease in accuracy.

How to control the color development of the standard song? (Only for double antibody sandwich ELISA kit)

The color development time of 5-30 minutes is the empirical range. For each specific experiment, the approximate color development time can be determined according to the following conditions:

1) Visual observation: When the S5 hole of Biaoqu has light blue and the Blank hole has no obvious blue, it can be terminated;

2) Instrument judgment: When the OD value of Biaoqu S1 hole reaches 0.5-0.7, the OD value of S5 hole reaches 0.05-0.08, and the OD value of Blank hole is less than 0.05 at a wavelength of about 630 nm, it can be terminated;

3) High-sensitivity series kits require strict control of the color development time due to their higher sensitivity, which can be appropriately shortened compared with ordinary kits.

For uncovered matters, please call ClusterTech's support hotline 400-6721-600 for more help.

Four, analysis

#### 1. Result calculation

Calculate the average OD value of the standard and the sample, and then subtract the OD value of the zero-concentration standard.

Taking the concentration of the standard substance as the abscissa and the OD value as the ordinate, the computer software was used to perform regression fitting to generate a standard curve. Regression analysis determines the best fit curve. By fitting the logarithm of the concentration value and the OD value, the standard curve can be linearized. This process may be able to get more sample concentrations, but the accuracy of the data will be reduced.

Note: The final concentration of the highest concentration point of the standard curve is 2,000 pg /ml.

If the serum/plasma sample is diluted according to the instructions, the final dilution factor is 20. If the sample has been diluted in other ways, please multiply by the corresponding dilution factor when calculating the sample concentration.

#### 2. Typical data

For each test, a standard curve must be established for each plate. The following standard curve is for reference only.

pg/ml O.D. Average Corrected

0.00 0.085 0.090 0.088

31.25 0.137 0.144 0.141 0.057

62.50 0.181 0.184 0.183 0.110

125.00 0.285 0.283 0.284 0.197

250.00 0.486 0.483 0.485 0.397

500.00 0.864 0.835 0.850 0.762

1000.00 1.522 1.486 1.504 1.417

2000.00 2.506 2.509 2.508 2.420

### 3. Sensitivity

The lowest detectable concentration of human Fas is 3.15 pg/ml (average of 6 independent experiments).

The average value of the OD of 10 zero standard concentrations plus twice the SD is calculated to calculate the lowest detectable concentration.

### 4. Precision

#### Microplate internal precision

The measurement was repeated 20 times in 3 samples of known concentration in the microtiter plate to evaluate the precision in the microtiter plate.

#### Precision between microplates

The test was repeated 6 times between 3 samples of known concentration in the microtiter plate to evaluate the precision between the microtiter plates.

#### Precision within microplates Precision between microplates

Sample 1 2 3 1 2 3

20 20 20 6 6 6

Average (pg/ml) 266.2 544.1 1045.4 266.3 538.4 1052.5

Standard deviation 10.5 15.3 30.8 13.4 25.4 33.6

Coefficient of variation (%) 3.9 2.8 2.0 5.0 4.7 3.2

### 5. Recovery rate

Three different concentrations of human Fas were added to 5 healthy human sera, and the serum without human Fas was used as a background, and the recovery rate was calculated. The recovery rate ranges from 95% to 106%, with an average recovery rate of 102%.

#### 6. Dilution linearity

High concentrations of human Fas were added to 5 healthy human serums, and serial dilutions were made within the kinetic range of the standard curve to evaluate the linearity of the test.

Average (%)	Range (%)
-------------	-----------

1:2	100 98-105
-----	------------

1:4	102 99-106
-----	------------

1:8	99 95-101
-----	-----------

1:16	97 92-103
------	-----------

#### 7. Calibration

The standard of this kit is high-purity recombinant human Fas calibrated by LinkTech.

#### 8. Sample value

This kit was used to test 30 serum samples from healthy volunteers. The volunteers' medication history is unknown.

sample

Type Test sample

Quantity Concentration range

(ng/ml) Measurable percentage

(%) Average concentration of measurable sample

(ng/ml)

Serum 30 2.0-17.4 100 6.7

Note: This sample value range is not a physiological value range. The concentration range of healthy human samples varies with species, sample preparation, testing personnel, and equipment. The above data is for reference only.

#### 9. Specificity

This kit recognizes natural and recombinant human Fas. The following factors were specifically evaluated, and no obvious cross-reactivity and interference effects

were observed.

Human mouse rat

ANG

AR

CNTF

B-ECGF

EGF

EPO

Fas Ligand

FGF acidic

FGF basic

FGF-4

FGF-5

FGF-6

G-CSF GM-CSF

HGF

IFN- $\gamma$

IL-1 $\beta$

IL-2

IL-4

IL-5

IL-6

IL-8

IL-10

IL-12

TNF-  $\alpha$

VEGF GM-CSF

IL-1  $\beta$

IL-2

IL-4

IL-6

IL-10

IL-17A

LIF

MIP-1  $\alpha$

MIP-1  $\beta$

SCF

TNF-  $\alpha$  IFN-  $\gamma$

IL-1  $\beta$

IL-4

IL-6

IL-10

TNF-  $\alpha$

#### 10. Summary of detection steps

1) Prepare all reagents and serially diluted standards. Add 300  $\mu$ l  $1 \times$  lotion to the slats and let stand and soak for 30 seconds.

2) Add 100  $\mu$ l of standard product diluted 2 times to the standard well.

Add 100  $\mu$ l  $1 \times$  detection buffer or culture medium to the blank wells.

3) Serum/plasma: add 100  $\mu$ l of pre-diluted sample to the sample well.

Cell culture supernatant: add 100  $\mu$ l of cell culture supernatant to the sample well. (For sample dilution, please refer to "Sample Preparation" on page 6).

4) Add 50  $\mu$ l 1:100 diluted detection antibody to each well. Steps 2, 3, and 4 are completed within 15 minutes.

5) Seal the membrane and incubate at room temperature for 2 hours. Wash 6 times.

- 6) Add 100  $\mu$ l 1:100 diluted horseradish peroxidase-labeled streptavidin to each well.
- 7) Seal the membrane and incubate at room temperature for 45 minutes. Wash 6 times.
- 8) Add 100  $\mu$ l chromogenic substrate to each well, protect from light, and incubate at room temperature for 5-30 minutes.
- 9) Add 100  $\mu$ l stop solution to each well.
- 10) Within 30 minutes, detect the OD value at a wavelength of 450 nm, with a reference wavelength of 570 nm or 630 nm.