FasL (rat) ELISA Kit

(Catalog # K3330-100, 100 assays; Store at -20^{0}C)

I. Introduction:

BioVision's rat FasL ELISA Kit is based on the standard sandwich enzyme-linked immunosorbent assay technology. This assay employs a monoclonal antibody form mouse specific for FasL coated on a 96-well plate. Standards (NSO, Q127-L278) and test samples are added to the wells and FasL present in a sample is bound to the wells by the immobilized antibody. A biotinylated detection polyclonal antibody from goat specific for FasL is added subsequently. After washing away the unbound biotinylated antibody with PBS or TBS buffer, Avidin-Biotin-Peroxidase Complex is added to the wells. The wells are again washed with PBS or TBS buffer to remove the unbound conjugates. HRP substrate TMB is used to visualize the HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue color product that changes into yellow after adding acidic stop solution. The density of yellow color is proportional to the rat FasL captured onto the plate. This ELISA kit shows no cross-reactivity with other relevant proteins. Detection Range: 31.2 pg/ml – 2000 pg/ml. Sensitivity: < 10 pg/ml.

II. Application:

Quantitative protein detection, establishing normal range etc.

III. Specificity:

Natural and recombinant rat FasL.

IV. Sample Type:

- Serum & plasma (heparin, EDTA, citrate)
- Cell culture supernatants

V. Kit Contents:

Components	K3330-100	Part No.
FasL Microplate coated with anti-rat Ab against FasL, 96 wells	12 stripsx8 wells	K3330-100-1
Lyophilized recombinant rat FasL standard (10 ng/vial)	2 vials	K3330-100-2
Biotinylated anti-rat FasL antibody	130 µl	K3330-100-3
Avidin-Biotin-Peroxidase Complex (ABC)	130 µl	K3330-100-4
Sample diluent buffer	30 ml	K3330-100-5
Antibody diluent buffer	12 ml	K3330-100-6
ABC diluent buffer	12 ml	K3330-100-7
TMB color developing agent (Colorless)	10 ml	K3330-100-8
TMB stop solution	10 ml	K3330-100-9

VI. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm.
- Absorbent paper.
- Adjustable pipettes and pipette tips. Multichannel pipettes are recommended in the condition of large amount of samples in the detection.
- Washing buffer (neutral PBS or TBS).
  - Preparation of 0.01M TBS: Add 1.2 g Tris, 8.5 g NaCl; 450 µl of purified acetic acid or 700 µl of concentrated hydrochloric acid to 1000 ml H_2O and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.
  - Preparation of 0.01 M PBS: Add 8.5 g sodium chloride, 1.4 g Na_2HPO_4 and 0.2 g NaH_2PO_4 to 1000 ml distilled water and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.

VII. Storage Conditions and Reagent Preparation:

Store kit at 4^{o}C for 6 months or at -20^{o}C for 12 months. Avoid repeated freeze-thaw cycles. Spin tubes briefly to bring down all components to the bottom of tubes.

- **Reconstitution of the rat FasL standard**: Two vials of FAS-L standard (10 ng per vial) are included in each kit. Use one vial for each experiment. Prepare 10,000 pg/ml of rat FAS-L standard solution by adding 1ml of sample diluent buffer into one of the vials. Keep the tube at room temperature for 10 min. and mix thoroughly. Prepare 2000 pg/ml of rat FasL standard solution by adding 0.2 ml of the 10 ng/ml FAS L Standard solution into 0.8 ml sample diluent buffer and mix thoroughly. Label 6 Eppendorf tubes with 1000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml, & 31.2 pg/ml respectively. Aliquot 0.3 ml of the sample diluent buffer into each tube. Add 0.3 ml of the 2000 pg/ml FasL 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 hrs. The 10 ng/ml standard solution should be stored at 4°C for up to 12 hrs, or at -20°C for up to 48 hrs. Avoid repeated freeze-thaw cycles.
- **Preparation of biotinylated anti-rat FasL antibody working solution**: Dilute 1:100 with the antibody diluent buffer and mix thoroughly. Prepare 0.1 ml of FasL antibody working solution for each well. Solution should be prepared no more than 2 hrs prior to the experiment.
- **Preparation of Avidin-Biotin-Peroxidase Complex (ABC) working solution**: Dilute 1:100 with the ABC dilution buffer and mix thoroughly. Prepare 0.1 ml of ABC working solution for each well. Solution should be prepared no more than 1 hr prior to the experiment.

VIII. Sample Preparation and Storage:

Centrifuge cell culture supernates to remove particulates, assay immediately or aliquot and store at -20°C. Allow the serum to clot in a serum separator tube (about 4 hrs) at room temperature. Centrifuge at approximately 1500 X g for 15 min. Analyze the serum immediately or aliquot and store frozen at -20°C. Collect plasma using heparin or EDTA or citrate as an anticoagulant. Centrifuge for 15 min. at 1500 x g within 30 min. of collection. For eliminating platelet, we suggest to further centrifuge at 10000 X g for 10 min. at 2-8°C. Analyze

immediately or aliquot and store frozen at -20°C. Analyze immediately or aliquot and store frozen at -20°C. Citrate is not recommended as the anticoagulant.

Notes:

a. Store samples to be assayed within 24 hrs. at 2-8°C. For long-term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.

b. Sample dilution guidelines: 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 sample must be well mixed with the diluents buffer. The following is a guideline for sample dilution. Several trials may be necessary in practice. For high target protein concentration (20-200 ng/ml): dilute 1:100. For medium target protein concentration (2-20 ng/ml): dilute 1:10. For low target protein concentration (31.2-2000 pg/ml): dilute 1:2. For very low target protein concentration (≤31.2 pg/ml). No dilution necessary or dilute 1:2.

IX. Assay Protocol:

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. Don't let 96-well plate dry, as it will inactivate active components on plate.

1. Aliquot 0.1ml per well of the 2000 pg/ml, 1000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml, & 31.2 pg/ml rat FasL 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 rat cell culture supernates, serum or plasma to each empty well. See "Sample Dilution Guideline" for details.
   Notes:
   a. We recommend that each rat FasL standard solution and each sample is measured in duplicate.
   b. We recommend doing a pilot experiment using standards and a small number of samples to inspect the validity of experiment operation and the appropriateness of sample dilution proportion.
2. Seal the plate with the cover and incubate at 37°C for 90 min. 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.
3. Add 0.1ml of biotinylated anti-rat FasL antibody working solution into each well and incubate the plate at 37°C for 60 min. Wash plate 3 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. (Plate Washing Method: 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 min. 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.)
4. Add 0.1ml of prepared ABC working solution into each well and incubate the plate at 37°C for 30 min. Wash plate 5 times with 0.01M TBS or 0.01 M 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. (See Step 3 for plate washing method).
5. Add 90µl of prepared TMB color developing agent into each well and incubate plate at 37°C in dark for 20-25 min.
   Note: For reference only, the optimal incubation time should be determined by end user. The shades of blue can be seen in the wells with the four most concentrated rat FAS-L standard solutions; the other wells show no obvious color.
6. Add 0.1ml of prepared TMB stop solution into each well. The color changes into yellow immediately.
7. Read the O.D. absorbance at 450 nm in a microplate reader within 30 min. after adding the stop solution.
8. Calculation: Relative $O.D._{450}$ = $O.D._{450}$ of each well – $O.D._{450}$ 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 rat FasL concentration of the samples can be interpolated from the standard curve. **Note:** if the samples were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

Typical Data Obtained from rat FasL
(TMB reaction incubated at 37°C for 20 min.)

Concentration(pg/ml)	0	31.2	62.5	125	250	500	1000	2000
O.D.	0.029	0.066	0.110	0.181	0.372	0.688	1.516	2.285

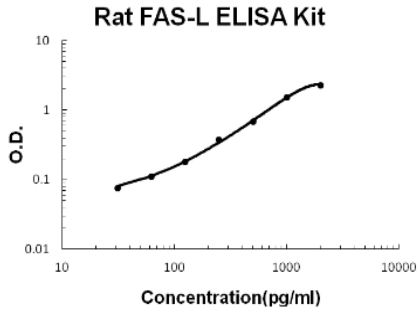

Figure: Standard Curve: This standard curves is for demonstration only. A standard curve must be run with each assay.

X. RELATED PRODUCTS:

FasL (mouse) ELISA Kit (K3331)
Fas Ligand Antibody (Clone I-6060) (3330)
FasL Blocking Peptide (3345RBP)
FasL (human) ELISA Kit (K4765)
FasL Antibody (3345R)

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