Datasheet for ABIN624972

FAS ELISA Kit

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Overview

Quantity:	96 tests
Target:	FAS
Reactivity:	Human
Method Type:	Sandwich ELISA
Application:	ELISA

Product Details

Purpose:	Human Fas (TNFRSF6/Apo-1) ELISA Kit for cell and tissue lysate samples.
Sample Type:	Cell Lysate, Tissue Lysate
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	The antibody pair provided in this kit recognizes human Fas.
Sensitivity:	5 pg/mL
Characteristics:	• Strip plates and additional reagents allow for use in multiple experiments • Quantitative protein detection • Establishes normal range • The best products for confirmation of antibody array data
Components:	• Pre-Coated 96-well Strip Microplate • Wash Buffer • Stop Solution • Assay Diluent(s) • Lyophilized Standard

Product Details

	• Biotinylated Detection Antibody • Streptavidin-Conjugated HRP • TMB One-Step Substrate
Material not included:	• Distilled or deionized water • Precision pipettes to deliver 2 µL to 1 µL volumes • Adjustable 1-25 µL pipettes for reagent preparation • 100 µL and 1 liter graduated cylinders • Tubes to prepare standard and sample dilutions • Absorbent paper • Microplate reader capable of measuring absorbance at 450nm • Log-log graph paper or computer and software for ELISA data analysis • Cell lysate buffer

Target Details

Target:	FAS
Alternative Name:	Fas (FAS Products)
Background:	Fas (APO-1 or CD95) is a cell-surface receptor that transduces apoptotic signals from Fas ligand (FasL). Fas and Fas Ligand (FasL) belong to the TNF superfamily and are type I and type II transmembrane proteins, respectively. Fas and FasL have been observed as soluble molecules in addition to their membraneassociated forms. Fas is expressed to a large extent on activated T and B lymphocytes, and on malignant lymphoid cells. The Human Fas ELISA (Enzyme-Linked Immunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of human Fas cell lysate and tissue lysate. This assay employs an antibody specific for human Fas coated on a 96-well plate. Standards and samples are pipetted into the wells and Fas present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-human Fas antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of Fas bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm. Reproducibility: Intra-Assay: CV<10% Inter-Assay: CV<12%.
Gene ID:	355
UniProt:	P25445
Pathways:	p53 Signaling, Apoptosis, Production of Molecular Mediator of Immune Response, Positive

Target Details

	Regulation of Endopeptidase Activity

Application Details

Sample Volume:	100 µL
Plate:	Pre-coated
Protocol:	1. Prepare all reagents, samples and standards as instructed in the manual. 2. Add 100 µL of standard or sample to each well. 3. Incubate 2.5 h at RT or O/N at 4 °C. 4. Add 100 µL of prepared biotin antibody to each well. 5. Incubate 1 h at RT. 6. Add 100 µL of prepared Streptavidin solution to each well. 7. Incubate 45 min at RT. 8. Add 100 µL of TMB One-Step Substrate Reagent to each well. 9. Incubate 30 min at RT. 10. Add 50 µL of Stop Solution to each well. 11. Read at 450 nm immediately.
Reagent Preparation:	1. Bring all reagents and samples to room temperature (18 - 25 °C) before use. 2. Sample dilution: Tissue lysate and cell lysate sample should be diluted at least 5-fold with 1x Sample Diluent Buffer. 3. Sample Diluent Buffer (Item D) and Assay Diluent (Item E) should be diluted 5-fold with deionized or distilled water before use. 4. Preparation of standard: Briefly spin the vial of Item C. Add 400 µL 1x Sample Diluent Buffer (Item D, Sample Diluent Buffer should be diluted 5-fold with deionized or distilled water) into Item C vial to prepare a 50 ng/mL standard. Dissolve the powder thoroughly by a gentle mix. Add 40 µL Fas standard from the vial of Item C, into a tube with 960 µL Sample Diluent Buffer to prepare a 2,000 pg/mL stock standard solution. Pipette 400 µL 1x Sample Diluent Buffer into each tube. Use the stock standard solution to produce a dilution series . Mix each tube thoroughly before the next transfer. 1x Sample Diluent Buffer serves as the zero standard (0 pg/mL). 40 µL standard + 960 µL 200 µL 200 µL 200 µL 200 µL 200 µL 200 µL 2,000 666.7 222.2 74.07 24.69 8.23 2.74 0 pg/mL pg/mL pg/mL pg/mL pg/mL pg/mL pg/mL 5. If the Wash Concentrate (20x) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1x Wash Buffer. 6. Briefly spin the Detection Antibody vial (Item F) before use. Add 100 µL of 1x Assay Diuent into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4 °C for 5 days). The detection antibody concentrate should be

	diluted 80-fold with 1x Assay Diuent and used in step 4 of Part VI Assay Procedure. 7. Briefly spin the HRP-Streptavidin concentrate vial (Item G) before use. HRP-Streptavidin concentrate should be diluted 640-fold with 1x Assay Diuent. For example: Briefly spin the vial (Item G) and pipette up and down to mix gently . Add 25 µL of HRP-Streptavidin concentrate into a tube with 16 ml 1x Assay Diluent to prepare a 640-fold diluted HRP- Streptavidin solution (don't store the diluted solution for next day use). Mix well. 8. Cell lysate buffer should be diluted 2-fold with deionized or distilled water (for cell lysate and tissue lysate).
Assay Procedure:	1. Bring all reagents and samples to room temperature (18 - 25 °C) before use. It is recommended that all standards and samples be run at least in duplicate. 2. Add 100 µL of each standard (see Reagent Preparation step 2) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or over night at 4 °C with gentle shaking. We recommend using 50-500 myg/mL of total protein for lysate sample. The amount of sample used depends on the abundance of target protein. More of the sample can be used if signals are too weak. If signals are too strong, the sample can be diluted further. 3. Discard the solution and wash 4 times with 1x Wash Solution. Wash by filling each well with Wash Buffer (300 myl) using a multi-channel Pipette or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels. 4. Add 100 µL of 1x prepared biotinylated antibody (Reagent Preparation step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking. 5. Discard the solution. Repeat the wash as in step 6. Add 100 µL of prepared Streptavidin solution (see Reagent Preparation step 7) to each well. Incubate for 45 minutes at room temperature with gentle shaking. 7. Discard the solution. Repeat the wash as in step 8. Add 100 µL of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking. 9. Add 50 µL of Stop Solution (Item I) to each well. Read at 450 nm immediately.
Calculation of Results:	Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points. Typical Data: These standard curves are for demonstration only. A standard curve must be run with each assay. Sample Diluent Buffer Human Fas concentrations (pg/mL) O D =4 50 n m 0.01 0.1 1 10 1 10 100 1,000 10,000

Application Details

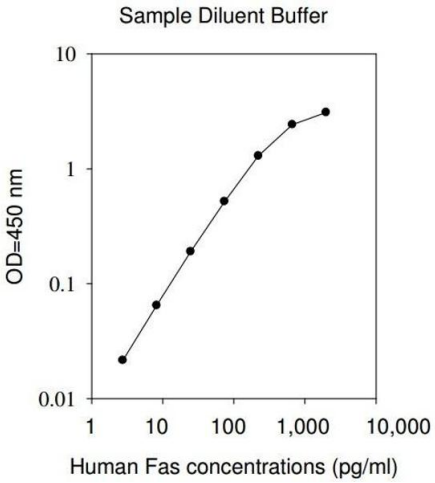
	Sensitivity: The minimum detectable dose of Fas is typically less than 5 pg/mL. Recovery: Recovery was determined by spiking various levels of human Fas into human tissue lysate and cell lysate. Mean recoveries are as follows: Sample Type Average % Recovery Range (%) Tissue lysate 89.79 82-104 Cell lysate 92.28 83-105 Linearity: Sample Type Tissue Cell Lysate lysate 1:2 Average % of 90 91 Expected Range (%) 82-103 81-104 1:4 Average % of 93 92 Expected Range (%) 83-105 82-105 Reproducibility: Intra-Assay: CV<10 % Inter-Assay: CV<12 %
Assay Precision:	Intra-Assay: CV< 10 % Inter-Assay: CV< 12 %
Restrictions:	For Research Use only

Handling

Handling Advice:	Avoid repeated freeze-thaw cycles.
Storage:	-20 °C
Storage Comment:	The entire kit may be stored at -20°C for up to 1 year from the date of shipment. Avoid repeated freeze-thaw cycles. The kit may be stored at 4°C for up to 6 months. For extended storage, it is recommended to store at -80°C.
Expiry Date:	6 months

Publications

Product cited in:	Szabò, Gulbins, Apfel, Zhang, Barth, Busch, Schlottmann, Pongs, Lang: "Tyrosine phosphorylation-dependent suppression of a voltage-gated K+ channel in T lymphocytes upon Fas stimulation." in: **The Journal of biological chemistry**, Vol. 271, Issue 34, pp. 20465-9, (1996) (PubMed). Mapara, Bargou, Zugck, Döhner, Ustaoglu, Jonker, Krammer, Dörken: "APO-1 mediated apoptosis or proliferation in human chronic B lymphocytic leukemia: correlation with bcl-2 oncogene expression." in: **European journal of immunology**, Vol. 23, Issue 3, pp. 702-8, (1993) (PubMed).



ELISA

Image 1.