Datasheet for ABIN625243

STAT1 ELISA Kit

7 Images

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Overview

Quantity:	96 tests
Target:	STAT1
Binding Specificity:	pSer727, total
Reactivity:	Human, Mouse
Method Type:	Sandwich ELISA
Application:	ELISA

Product Details

Purpose:	Human/Mouse Phospho-Stat 1 (S727) and Total Stat 1 ELISA Kit. This assay semi-quantitatively measures phophorylated Stat 1 (Ser727) and Total Stat 1 in lysate samples.
Sample Type:	Cell Lysate, Tissue Lysate
Analytical Method:	Semi-Quantitative
Detection Method:	Colorimetric
Specificity:	The antibody pair provided in this kit recognizes human and mouse Phospho-Stat1 (pSer727) + pan Stat1.
Characteristics:	• Simultaneously measure Phosphorylated protein and pan protein in one experiment (for normalization purpose) • Screen numerous different cell lysates without performing a Western Blot analysis • Minimal hands-on time, convenient, and non-radioactive material
Components:	• Pre-Coated 96-well Strip Microplate • Wash Buffer

Product Details

	• Anti-Phospho Antibody • Anti-Pan Antibody • HRP-Conjugated Secondary Antibody • Streptavidin-Conjugated HRP • Assay Diluent • TMB One-Step Substrate • Stop Solution • Lysis Buffer • Positive Control Sample
Material not included:	• Distilled or deionized water • 100 mL and 1 liter graduated cylinders • Tubes to prepare sample dilutions • Protease and Phosphatase inhibitors • Precision pipettes to deliver 2 µL to 1 mL volumes • Adjustable 1-25 mL pipettes for reagent preparation • Benchtop rocker or shaker • Microplate reader capable of measuring absorbance at 450 nm

Target Details

Target:	STAT1
Alternative Name:	Stat1 (STAT1 Products)
Background:	Stat1-S727
Gene ID:	6772
UniProt:	P42224
Pathways:	JAK-STAT Signaling, RTK Signaling, Interferon-gamma Pathway, Response to Growth Hormone Stimulus, Cellular Response to Molecule of Bacterial Origin, Positive Regulation of Endopeptidase Activity, Hepatitis C, CXCR4-mediated Signaling Events

Application Details

Sample Volume:	100 µL
Plate:	Pre-coated
Protocol:	1. Prepare all reagents and samples as instructed in the manual. 2. Add 100 µL of sample or positive control to each well. 3. Incubate 2.5 h at RT or O/N at 4 °C.

	4. Add 100 µL of prepared primary antibody to each well. 5. Incubate 1 h at RT. 6. Add 100 µL of prepared 1X HRP-Streptavidin to each well. 7. Incubate 1 h 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. Item E, Assay Diluent should be diluted 5-fold with deionized or distilled water before use. 3. Preparation of Positive Control: Briefly spin the Positive Control vial of Item K. Add 700 µL 1x Assay Diluent (Item E, Assay Diluent should be diluted 5-fold with deionized or distilled water before use) into Item K vial to prepare a Positive Control (P-1). Phospho-Stat1 (Ser727) and pan Stat1 ELISA Kit Protocol 6 Dissolve the powder thoroughly by a gentle mix (it can be removed by centrifuge if any precipitate in the solution is found). Pipette 260 µL 1x Assay Diluent into each tube. Use the P-1 to produce a dilution series (shown below). Mix each tube thoroughly before the next transfer. 1x Assay Diluent serves as the background. 4. 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. 5. Briefly spin the biotinylated antibody (Item C) before use. Add 100 µL of 1x Assay Diluent into the vial to prepare a biotinylated antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4 °C for 5 days or at - 80 °C for one month). The biotinylated Stat1 antibody should be diluted 55-fold with 1x Assay Diuent and used in step 4 of Part VII Assay Procedure. 6. Briefly spin the HRP-Streptavidin concentrate vial (Item G) and pipette up and down to mix gently before use. HRP-Streptavidin P-1 P-2 P-3 P-4 0 130 µL 700 µL 1x Assay Diluent + Item K vial 130µ l 130 µL Phospho-Stat1 (Ser727) and pan Stat1 ELISA Kit Protocol 7 concentrate should be diluted 40 fold or 200 fold (see “step 6” in page 8 for detail) with 1x Assay Diluent. For example: Briefly spin the vial (Item G) and pipette up and down to mix gently . Add 150µl of HRP-Streptavidin concentrate into a tube with 6 mL 1x Assay Diluent to prepare a 40-fold diluted HRP-Streptavidin solution (don’t store the diluted solution for next day use). Mix well. 7. Cell Lysate Buffer should be diluted 2-fold with deionized or distilled water before use (recommend to add protease and phosphatase inhibitors). VII.
Sample Preparation:	Cell lysates - Rinse cells with PBS, making sure to remove any remaining PBS before adding the Cell Lysate Buffer. Solubilize cells at 4 x 107 cells/mL in 1x Cell Lysate Buffer (we recommend

	adding protease and phosphatase inhibitors to Cell Lysate Buffer prior to sample preparation). Pipette up and down to resuspend and incubate the lysates with shaking at 2 - 8° C for 30 minutes. Microcentrifuge at 13,000 rpm for 10 minutes at 2 - 8° C, and transfer the supernates into a clean test tube. Lysates should be used immediately or aliquoted and stored at -70 °C. Avoid repeated Phospho-Stat1 (Ser727) and pan Stat1 ELISA Kit Protocol 5 freeze-thaw cycles. Thawed lysates should be kept on ice prior to use. For the initial experiment, we recommend to do a serial dilution testing such as 5-fold and 100-fold dilution for your cell lysates with Assay Diluent (Item E) before use. Note: The fold dilution of sample used depends on the abundance of phosphorylated proteins and should be determined empiricallys. More of the sample can be used if signals are too weak. If signals are too strong, the sample can be diluted further. Cell Lysate Buffer should be diluted 2-fold with deionized or distilled water before use (recommend to add protease and phosphatase inhibitors).
Assay Procedure:	1. Bring all reagents to room temperature (18 - 25 °C) before use. It is recommended that all samples or Positive Control should be run at least in duplicate. 2. Add 100 µL of each sample or positive control into appropriate wells (see the following 96 well microplate formate). Cover well with plate holder and incubate for 2.5 hours at room temperature or over night at 4 °C with shaking. 96 well microplate coated with phosphorylated and pan antibodies: Phospho-Stat1 (Ser727) and pan Stat1 ELISA Kit Protocol 8 Anti-Stat1 (Ser 727) Anti-pan Stat1 3. Discard the solution and wash 4 times with 1x Wash Solution. Wash by filling each well with Wash Buffer (300 µL) 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 prepared 1x biotinylated Stat1 antibody (Reagent Preparation step 5) to each well. Incubate for 1 hour at room temperature with shaking. 5. Discard the solution. Repeat the wash as in step3. 6. Add 100 µL of 40 fold diluted HRP-Streptavidin solution (see Reagent Preparation step 7) to each well coated with anti- Stat1 (Ser 727) on the left side (red marker, see Assay Procedure step1). Add 100 µL of 200 fold diluted HRP- 1 2 3 4 5 6 7 8 9 10 11 12 A B C D E F G H Phospho-Stat1 (Ser727) and pan Stat1 ELISA Kit Protocol 9 Streptavidin solution to each well coated with anti-pan Stat1 on the right side (black marker). Incubate for 1 hour at room temperature with shaking. 7. Discard the solution. Repeat the wash as in step3. 8. Add 100 µL of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30

Application Details

	minutes at room temperature in the dark with shaking. 9. Add 50 µL of Stop Solution (Item I) to each well. Read at 450 nm immediately.
Calculation of Results:	ELISA data analysis: Average the duplicate readings for each sample or positive control. i. Positive Control A431 cells were treated with recombinant human EGF at 37 °C for 20 min. Solubilize cells at 4 x 107 cells/mL in lysis buffer. Serial dilutions of lysates were analyzed in this ELISA. Please see step 3 of Part VI. Reagent Preparation for detail. O D = 4 5 0 n m 0.0 0.5 1.0 1.5 2.0 2.5 Assay Diluent Positive control dilution series P-1 P-2 P-3 P-4 P-5 Phospho-Stat1 (Ser727) and pan Stat1 ELISA Kit Protocol 11 ii. Recombinant Human EGF Stimulation of A431 Cell Lines A431 cells were treated or untreated with 100 ng/mL recombinant human EGF for 10 min. Cell lysates were analyzed using this phosphoELISA and Western Blot. A). ELISA Phospho-Stat1 (Ser727) Total Stat1 O D =4 50 n m 0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 Untreated A431 EGF treated A431 B). Western-Blot Analysis hEGF 0 10 0 10 (Min) Anti-phospho-Stat1 Anti-Stat1 (Ser727) Phospho-Stat1 (Ser727) and pan Stat1 ELISA Kit Protocol 12 iii. SENSITIVITY The A431 cells were treated with 100 ng/mL recombinant human EGF for 20 minutes to induce phosphorylation of Stat1. Serial dilutions of lysates were analyzed in this ELISA and by Western blot. Immunoblots were incubated with anti-phospho-Stat1 (Ser 727). A). ELISA 50 1 0 2 0.4 0.8 0 (µg) O D =4 50 n m 0.0 0.2 0.4 0.6 0.8 1.0 1.2 1.4 1.6 B). Western-Blot Analysis 50 25 12.5 6.25 3.13 1.56 0.78 0.39 0 (µg) Phospho-Stat1 (Ser727) and pan Stat1 ELISA Kit Protocol 13 X.
Restrictions:	For Research Use only

Handling

Handling Advice:	Avoid repeated freeze- thaw cycles.
Storage:	-20 °C
Storage Comment:	Upon receipt, the kit should be stored at -20 °C. Please use within 6 months from the date of shipment. After initial use, Wash Buffer Concentrate (Item B), Assay Diluent (Item E), TMB One-Step Substrate Reagent (Item H), HRP-Streptavidin (Item G), Stop Solution (Item I) and Cell Lysate Buffer (Item J) should be stored at 4 °C to avoid repeated freeze-thaw cycles. Return unused wells to the pouch containing desiccant pack, reseal along entire edge and store at -20 °C. Reconstituted Positive Control (Item K) should be stored at -70 °C.
Expiry Date:	6 months

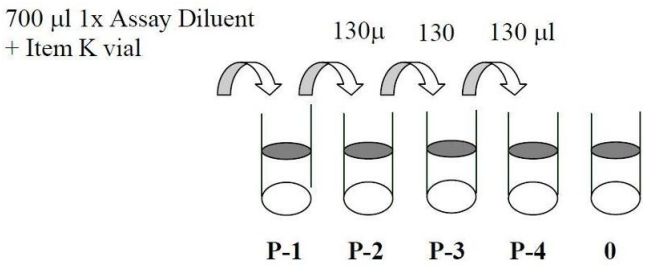

Image 1. This picture shows the preparation of the positive control.

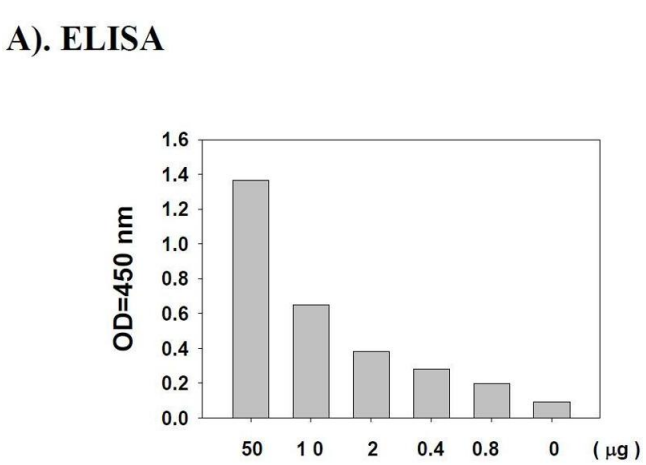

Image 2.

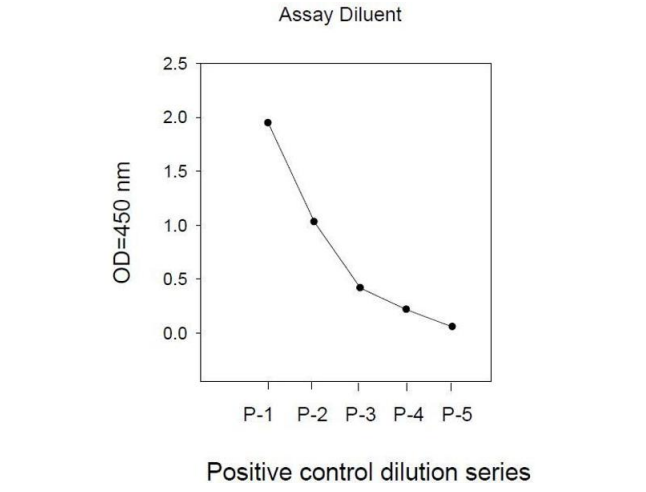

Image 3.

Please check the product details page for more images. Overall 7 images are available for ABIN625243.