Datasheet for ABIN625245

STAT3 ELISA Kit

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Overview

Quantity:	96 tests
Target:	STAT3
Binding Specificity:	pTyr705, total
Reactivity:	Human, Rat, Mouse
Method Type:	Sandwich ELISA
Application:	ELISA

Product Details

Purpose:	Human/Mouse/Rat Phospho-Stat 3 (Y705) and Total Stat 3 ELISA Kit. This assay semi-quantitatively measures phophorylated Stat 3 (Tyr705) and Total Stat 3 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, mouse and rat Phospho-Stat3 (pTyr705) + pan Stat3.
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:	STAT3
Alternative Name:	Stat3 (STAT3 Products)
Background:	Stat3-Y705
Gene ID:	6774
UniProt:	P40763
Pathways:	JAK-STAT Signaling, RTK Signaling, Interferon-gamma Pathway, Neurotrophin Signaling Pathway, Dopaminergic Neurogenesis, Response to Growth Hormone Stimulus, Carbohydrate Homeostasis, Stem Cell Maintenance, Hepatitis C, Protein targeting to Nucleus, Feeding Behaviour, CXCR4-mediated Signaling Events, Signaling of Hepatocyte Growth Factor Receptor

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. Assay Diluent (Item E) 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 500 µL of the prepared 1x Assay Diluent (Item E) into Item K vial to prepare Positive Control stock solution. Dissolve the powder thoroughly by a gentle mix (precipitate can be removed by centrifuging if present). Add 150 µL Positive Control stock solution from the vial of Item K, into a tube with 300 µL 1x Assay Diluent to prepare a Positive Control (1) (P(1)) (See i. Positive Control of part IX. TYPICAL DATA for a typical result on page 10). Pipette 300 µL 1x Assay Diluent into 4 other tubes. Use the Positive Control (1) to produce a dilution series (shown below). Mix each tube thoroughly before the next transfer. 1x Assay Diluent serves as the background (0). P (1) P (2) P (3) P (4) 0 150 µL 150 µL Positive Control stock solution 300 µL 1x Assay Diluent 150µ l 150 µL Phospho-Stat3 (Tyr705) and pan Stat3 ELISA Kit Protocol 7

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 detection antibody (Item C-1 or Item C-2) before use. Add 100 µL of 1x Assay Diluent 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 or at - 80 °C for one month). The anti-phospho-Stat3 (Tyr705) or biotinylated anti-pan-Stat3 antibody should be diluted 55-fold with 1x Assay Diuent and used in step 3 of Part VII Assay Procedure.

6. Briefly spin the HRP-conjugated anti-rabbit IgG (Item D-1) or HRP-streptavidin concentrate (Item G) before use. Pipette up and down to mix gently. HRP-conjugated anti-rabbit IgG concentrate should be diluted 2,000-fold and HRP-streptavidin concentrate should be diluted 80-fold with 1x Assay Diluent. For example: Briefly spin the vial (Item G) and pipette up and down to mix gently. Add 50 µL of HRP-streptavidin concentrate into a tube with 4.0 mL 1x Assay Diluent to prepare an 80-fold diluted HRP-streptavidin solution.

7. Cell Lysate Buffer should be diluted 2-fold with deionized or distilled water before use

	(recommend to add protease and phosphatase inhibitors). Phospho-Stat3 (Tyr705) and pan Stat3 ELISA Kit Protocol 8 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 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 50-fold dilution for your cell lysates with Assay Diluent (Item E) before use. Note: The optimal sample dilution fold will depend on the abundance of phosphorylated proteins and should be determined empirically. 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 (Item J) should be diluted 2-fold with deionized or distilled water before use (recommend to add protease and phosphatase inhibitors). Phospho-Stat3 (Tyr705) and pan Stat3 ELISA Kit Protocol 6
Assay Procedure:	1. Bring all reagents to room temperature (18 - 25 °C) before use. It is recommended that all samples and Positive Control should be run at least in duplicate. Add 100 µL of each sample or positive control into appropriate wells. Cover well with plate holder and incubate for 2.5 hours at room temperature or overnight at 4 °C with shaking. 2. Discard the solution and wash 4 times with 1x Wash Buffer 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. 3. Add 100 µL of prepared 1x rabbit anti-phospho-Stat3 (Tyr705) antibody or 1x biotinylated anti-pan-Stat3 (Reagent Preparation step 5) to appropriate wells. Incubate for 1 hour at room temperature with shaking. 4. Discard the solution. Repeat the wash as in step 2. 5. Add 100 µL of prepared 1X HRP-conjugated anti-rabbit IgG against rabbit anti-Stat3 (Tyr705) or 1X HRP-streptavidin against biotinylated anti-Stat3 (see Reagent Preparation step 6) to corresponding well. Incubate for 1 hour at room temperature with shaking. Phospho-Stat3 (Tyr705) and pan Stat3 ELISA Kit Protocol 9

Application Details

	6. Discard the solution. Repeat the wash as in step 2. 7. 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 shaking. 8. 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 well. 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 Cell Lysate Buffer. Serial dilutions of lysates were analyzed in this ELISA. Please see step 3 of Part VI Reagent Preparation for details. O D = 4 5 0 n m 0.0 0.2 0.4 0.6 0.8 1.0 1.2 Assay Diluent Positive control dilution series P-1 P-2 P-3 P-4 0 Phospho-Stat3 (Tyr705) and pan Stat3 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 20 min. Cell lysates were analyzed using this phosphoELISA and Western Blot. A). ELISA Anti-Stat 3 (Tyr705) Anti-pan Stat3 O D = 45 0 n m 0.0 0.5 1.0 1.5 2.0 hEGF treated A431 Untreated A431 B). Western-Blot Analysis hEGF 20 0 20 0 (Min) Anti-phospho-Stat3 Anti-Stat3 (Tyr705) Phospho-Stat3 (Tyr705) and pan Stat3 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 EGF R. Serial dilutions of lysates were analyzed in this ELISA and by Western blot. Immunoblots were incubated with anti-phospho-Stat3 (Tyr705). A) ELISA 20 6.7 2.2 0.74 0.25 0 (µg) O D =4 50 n m 0.0 0.2 .4 0.6 0.8 1.0 1.2 1.4 B). Western-Blot Analysis 50 25 12.5 6.25 3.13 1.56 0.78 0.39 0 (µg) Phospho-Stat3 (Tyr705) and pan Stat3 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.

Handling

Expiry Date:	6 months

Publications

Product cited in:	Romero, Holmgren-Holm, Grasa, Esteve, Remesar, Fernández-López, Alemany: "Modulation in Wistar rats of blood corticosterone compartmentation by sex and a cafeteria diet." in: **PLoS ONE**, Vol. 8, Issue 2, pp. e57342, (2013) (PubMed).

Images

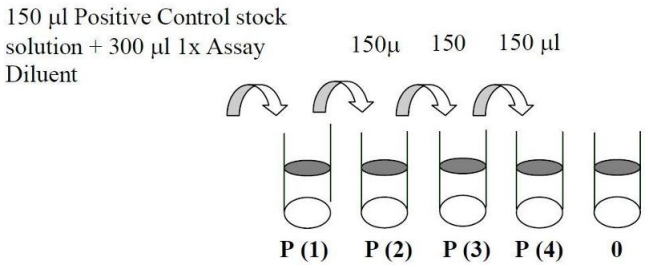

Image 1. This picture shows the preparation of the positive control.

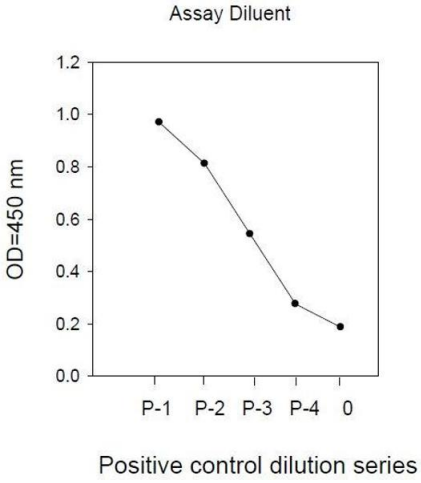

Image 2.

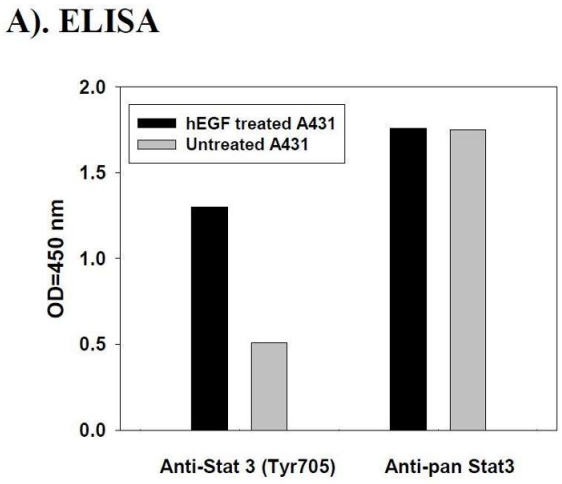

Image 3.

Please check the product details page for more images. Overall 6 images are available for ABIN625245.