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Product Information

Phospho-Stat1 (pTyr^{701}) and pan-Stat1 ELISA Kit
For detection of phospho-stat1 (pTyr^{701}) and pan-stat1 in human or mouse cell and tissue lysates

Catalog Number **RAB0443**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Phospho-STAT1 (Tyr^{701}) and Pan STAT1 ELISA (Enzyme-Linked Immunosorbent Assay) Kit is a very rapid, convenient, and sensitive assay kit that can monitor the activation or function of important biological pathways in cell lysates. By determining phosphorylated STAT1 in the experimental model system, pathway activation can be verified in the cell lysates. One can simultaneously measure numerous different cell lysates without spending excess time and effort in performing a Western blot analysis.

This Sandwich ELISA kit is an *in vitro* enzyme-linked immunosorbent assay for the measurement of human and mouse STAT1 (Tyr^{701}) and pan STAT1. The left side of a 96 well plate is coated with anti-STAT1 (Tyr^{701}) (distinguished by red marker) and the right side of the plate is coated with anti-pan STAT1 antibody (distinguished by black marker). Samples are pipetted into the wells, and phosphorylated STAT1 (left side) and pan STAT1 (right side) present in a sample are bound to the wells by the immobilized antibodies. The wells are washed and biotinylated anti-Stat1 is used to detect phosphorylated STAT1 (Tyr^{701}) or pan STAT1. After washing away unbound antibody, HRP-Streptavidin is pipetted into 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 STAT1 (Tyr^{701}) or pan STAT1 bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

Components

1. 2× Cell Lysate Buffer (Item J): 10 mL of 2× cell lysis buffer (does not include protease and phosphatase inhibitors).
2. 5× Assay Diluent (Item E2): 15 mL of 5× concentrated buffer. For diluting cell lysate samples, detection antibody (Item C-2), and HRP-Streptavidin concentrate.
3. HRP-conjugated Anti-Rabbit IgG Concentrate (Item G): 1 vials (200 µL) of 200× concentrated HRP-conjugated streptavidin.
4. Pan Stat1 Antibody (Item C/Item C2): 2 vials of biotinylated anti-Stat1 (1 vial is enough to assay half microplate).
5. Phosphorylation ELISA Stop Solution (Item I): 8 mL of 0.2 M sulfuric acid.
6. Capture Antibody-Coated Microplate (Item A): 96 wells (12 strips × 8 wells). Six strips, marked with red marker (provided on the left), are coated with anti-phospho-STAT1 (Tyr^{701}). The remaining 6 strips, marked with black marker (provided on the right), are coated with anti-pan-STAT1.
7. PhosphoELISA Lyophilized Positive Control Sample for Phospho-Stat1 (pTyr^{701}): 1 vial of lyophilized powder from A431 cell lysate.
8. TMB One-Step Substrate Reagent (Item H): 12 mL of 3,3′,5,5′-tetramethylbenzidine (TMB) in buffer solution.
9. 20× Wash Buffer Concentrate (Item B): 25 mL of 20× concentrated solution.

Reagents and Equipment Required but Not Provided.

1. Microplate reader capable of measuring absorbance at 450 nm
2. Protease and Phosphatase inhibitors
3. Shaker
4. Precision pipettes to deliver 2 µL to 1 mL volumes
5. Adjustable 1-25 mL pipettes for reagent preparation
6. 100 mL and 1 liter graduated cylinders
7. Absorbent paper
8. Distilled or deionized water
9. Log-log graph paper or computer and software for ELISA data analysis
10. Tubes to prepare the positive control or sample dilutions

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions
Sample Preparation
2× Cell Lysate Buffer should be diluted 2-fold with deionized or distilled water to yield 1× Cell Lysate Buffer (addition of protease and phosphatase inhibitors to 1× Cell Lysate Buffer is recommended prior to sample preparation).

Cell lysates - Rinse the cells with PBS, making sure to remove any remaining PBS before adding 1× Cell Lysate Buffer. Solubilize cells at 4 × 107 cells/mL in prepared Cell Lysate Buffer (Item J) (see Reagent Preparation step 3). Pipette up and down to resuspend the pellet. 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 supernatants 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, it is recommended to do a serial dilution, such as a 5-fold to 100-fold dilution, for the cell lysates with prepared Assay Diluent (Item E2) (see Reagent Preparation step 2) before use.

Note: The fold dilution of sample used depends 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.

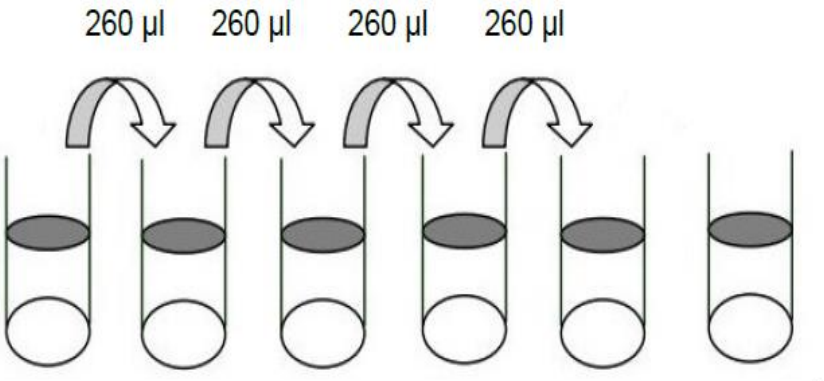
Reagent Preparation
1. Bring all reagents and samples to room temperature (18–25 °C) before use.

2. 5× Assay Diluent (Item E2) should be diluted 5-fold with deionized or distilled water before use.

3. Cell lysate buffer (Item J) should be diluted 2-fold with deionized or distilled water (for cell lysate and tissue lysate). The addition of protease and phosphatase inhibitors (not included) to the lysis buffer prior to use is recommended.

4. Preparation of Positive Control: Briefly spin the Positive Control Vial (Item K). Add 700 µL of prepared 1× Assay Diluent (Item E2) into Item K to prepare a Positive Control (P-1) solution. Gently mix the powder to allow it to dissolve thoroughly. If a precipitate is seen in the solution after mixing, this can be removed by a quick centrifugation of the positive control vial, and then pipetting the supernatant only for the assay. Pipette 260 µL of 1× Assay Diluent into each tube. Use the Positive Control (P-1) solution to produce a dilution series (see Figure 1). Mix each tube thoroughly before the next transfer. 1× Assay Diluent serves as the blank (P-0).

Figure 1.
Dilution Series for Positive Control



	P-1	P-2	P-3	P-4	P-5		Blank (P-0)
Diluent volume	Item K + 700 µl	260 µl	260 µl	260 µl	260 µl		260 µl

5. If the Wash Concentrate (20×) (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 1× Wash Buffer.

6. Preparation of biotinylated anti-Stat1 antibody: Briefly spin the vial of biotinylated anti-Stat1 (Item C-2). Add 100 µL of 1× Assay Diluent into the vial to prepare a pan detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4 °C for 5 days or at –70 °C for one month). The concentrate should then be diluted 55-fold with 1× Assay Diluent and used in step 4 of the Assay Procedure.

7. Preparation of HRP-Streptavidin: Briefly spin the vial of HRP-Streptavidin concentrate (Item G) before use. HRP-Streptavidin should be diluted 200-fold with 1× Assay Diluent and used in Procedure, step 7.

   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 10 mL of 1× Assay Diluent to prepare a 200-fold diluted HRP Streptavidin solution (don't store the diluted solution for next day use). Mix well.

Storage/Stability

Store the kit at –20 °C. It remains active for up to 1 year. Avoid repeated freeze-thaw cycles.

The reconstituted standard should be stored at –20 °C or –70 °C (–70 °C is recommended). Opened microplate strips or reagents may be store for up to 1 month at 2–8 °C. Return unused wells to the pouch containing desiccant pack and reseal along entire edge.

Procedure

1. Bring all reagents and samples to room temperature (18–25 °C) before use. It is strongly recommended to run all positive controls and samples in at least duplicate. It is also recommended to run the positive controls in singlet for each of the pan and phospho-specific antibodies.

2. Label removable 8-well strips as appropriate for the experiment.

3. Add 100 µL of positive control (see Reagent Preparation step 4) or sample into appropriate wells. Cover the wells and incubate for 2.5 hours at room temperature or overnight at 4 °C with gentle shaking.

4. Discard the solution and wash 4 times with 1× Wash Solution. Wash by filling each well with Wash Buffer (300 µL) using a multichannel pipette or autowasher. Complete removal of liquid at each step is essential for 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.

5. Add 100 µL of prepared 1× biotinylated anti-Stat1 antibody (see Reagent Preparation, step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking.

6. Discard the solution. Repeat the wash as in step 4.

7. Add 100 µL of prepared HRP-Streptavidin solution (see Reagent Preparation, step 7) to each well. Incubate for 1 hour at room temperature with gentle shaking.

8. Discard the solution. Repeat the wash as in step 4.

9. 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.

10. Add 50 µL of Stop Solution (Item I) to each well. Read at 450 nm immediately.

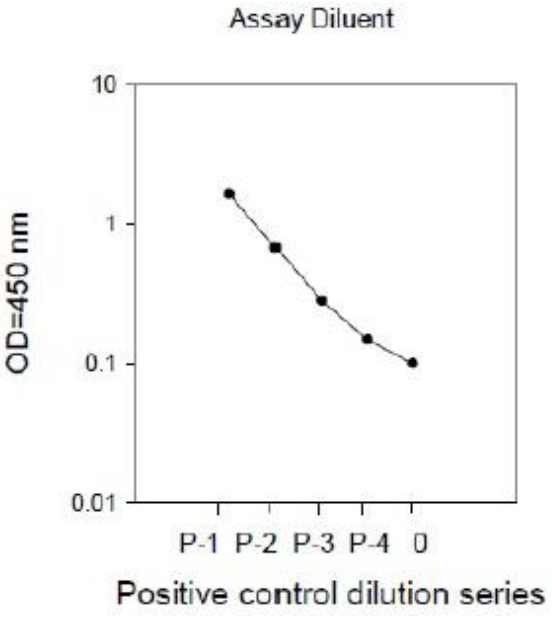
Results

Typical Data

Calculate the mean absorbance for each sample. Then, subtract the average zero (blank) optical density from each sample mean and set of singlet positive controls.

Positive Control

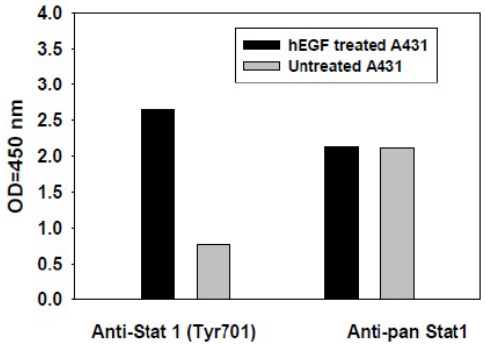
A431 cells were treated with recombinant human EGF at 37 °C for 20 minutes. Cells were solubilzed at 4×10^7 cells/mL in lysis buffer. Serial dilutions of lysates were analyzed with this ELISA. Please see Preparation, step 3 for details.



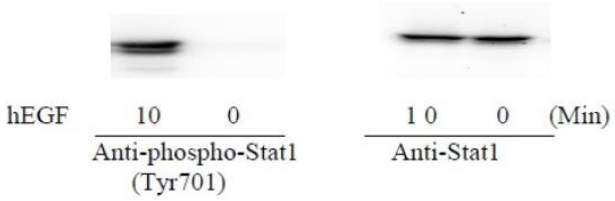
Recombinant Human EGF Stimulation of A431 Cell Lines

A431 cells were untreated or treated with 100 ng/mL recombinant human EGF for 10 minutes. Cell lysates were analyzed using this phosphoELISA and Western blot.

ELISA



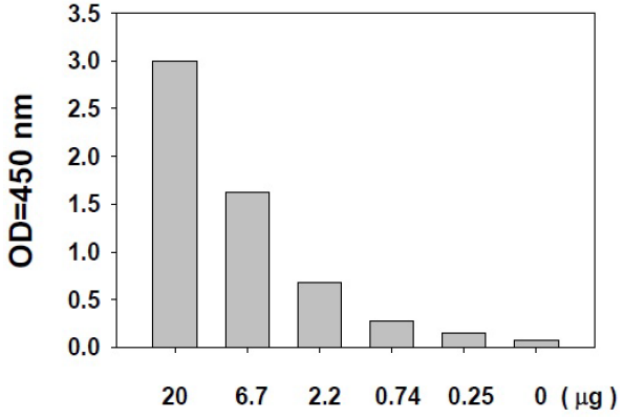
Western Blot Analysis



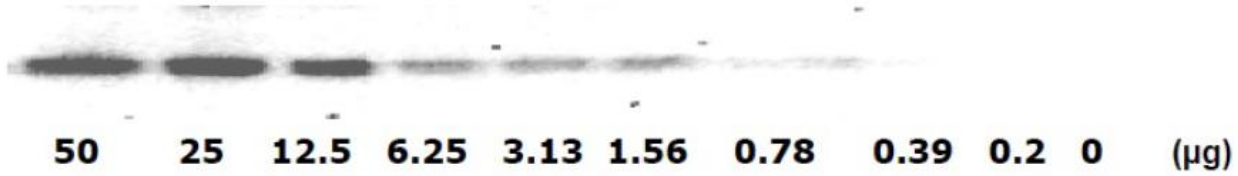
Sensitivity

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 with this ELISA and by Western blot. Immunoblots were incubated with antiphospho-Stat1 (Tyr^{701}).

ELISA



Western Blot



Appendix

Troubleshooting Guide

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standard dilution	Ensure a brief spin of Item C and dissolve the powder thoroughly with gentle mixing.
Low signal	Too brief incubation times	Ensure sufficient incubation time; Procedure, step 2 may change to over night
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Large CV	Inaccurate pipetting	Check pipettes
High background	Plate is insufficiently washed	Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed.
	Contaminated wash buffer	Make fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store the standard at <–20 °C after reconstitution, others at 4 °C. Keep substrate solution protected from light
	Stop solution	Stop solution should be added to each well before measurement.

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