Version 3a Last updated 29 October 2020

ab126457 - STAT1 (pY701) + total STAT1 ELISA Kit

For the semi-quantitative measurement of Human and Mouse phosphorylated STAT1 (Tyr701) and total STAT1 concentrations in cell lysates.

This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview

ab126457 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 protein in your experimental model system, you can verify pathway activation in your cell lysates. You 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 phospho-STAT1 (Tyr701) and total STAT1 (help normalize the results of phospho-STAT1 from different cell lysate being compared). An anti-pan STAT1 antibody has been coated onto a 96-well plate. Samples are pipetted into the wells and STAT1 present in a sample is bound to the wells by the immobilized antibody and the wells are washed. In select wells, rabbit anti-phospho-STAT1 (Tyr701) antibody is added to detect phosphorylated STAT1. In the remaining wells, biotinylated anti-STAT1 antibody is used to detect pan STAT1. After washing away unbound antibody, HRP-conjugated anti-rabbit IgG or 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 (Tyr701) 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.

2. Protocol Summary

Prepare all reagents, samples, and standards as instructed

Add 100 µl sample or positive control to each well. Incubate 2.5 hours at room temperature or overnight at 4°C with gentle shaking.

Add 100 µl prepared detection antibody to each well. Incubate 1 hour at room temperature with gentle shaking.

Add 100 µl prepared HRP-Conjugated solution. Incubate for 1 hour at room temperature with gentle shaking.

Add 100 µl TMB One-Step Substrate Reagent to each well. Incubate 30 minutes at room temperature.

Add 50 µl Stop Solution to each well. Read at 450 nm immediately.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Upon receipt, the kit should be stored at –20°C for up to 6 months from the date of shipment. Avoid repeated freeze-thaw cycles. For extended storage it is recommended to store at -80°C.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition
Pan STAT1 Microplate (12 x 8 wells) coated with anti-pan STAT1 antibody	96 wells	-20°C
Phospho Detection Antibody STAT1 (Tyr701)	1 vial	-20°C
Pan Detection Antibody STAT1: Biotinylated	1 vial	-20°C
HRP-conjugated anti-rabbit IgG 500X concentrate	25 µL	-20°C
Positive Control: lyophilized powder from A431 cell lysate	1 vial	-20°C
100X HRP-Streptavidin Conjugate	200 µL	-20°C
2X Cell Lysis Buffer	10 mL	-20°C
5X Assay Diluent	15 mL	-20°C
Stop Solution	8 mL	-20°C
20X Wash Buffer	25 mL	-20°C
TMB One-Step Substrate Reagent	12 mL	-20°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

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

8. Technical Hints

- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Make sure all buffers and solutions are at room temperature before starting the experiment.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Make sure you have the right type of plate for your detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on before starting the experiment.

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use.
- Prepare fresh reagents immediately prior to use.

9.1 Pan STAT1 Microplate (12 x 8 wells) coated with anti-pan STAT1 antibody

Ready to use as supplied. Once opened store for up to 1 month at -20°C. Return unused wells to the pouch containing desiccant pack, reseal along entire edge.

9.2 5X Assay Diluent

Dilute the 5X Assay Diluent 5-fold with deionized or distilled water. Mix gently and thoroughly.

Δ Note: Store for up to 1 month at 4°C.

9.3 2X Cell Lysis Buffer

Dilute the 2X Cell Lysis Buffer 2-fold with deionized or distilled water (for cell lysate and tissue lysate). We also recommend the addition of protease and phosphatase inhibitors (not included) to the lysis buffer prior to use. Mix gently and thoroughly.

Δ Note: Store for up to 1 month at 4°C.

9.4 20X Wash Buffer

If the 20X Wash Buffer contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 mL of 20X Wash Buffer into deionized or distilled water to yield 400 mL of 1X Wash Buffer.

Δ Note: Store for up to 1 month at 4°C.

9.5 Phospho Detection Antibody STAT1 (Tyr701)

Briefly spin the vial of Phopho Detection Antibody STAT1 (Tyr701). Add 100 µl of 1X Assay Diluent into the vial to prepare a phospho 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 concentrate should then be diluted 55-fold with 1x Assay Diluent for use in the assay procedure.

9.6 Pan Detection Antibody STAT1: Biotinylated

Briefly spin the vial of biotinylated Pan Detection Antibody STAT1. Add 100 µl of 1X 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 –80°C for one month). The concentrate should then be diluted 55-fold with 1x Assay Diluent for use in the assay procedure.

9.7 HRP-conjugated anti-rabbit IgG 500X concentrate

Briefly spin the vial before use. Dilute the required amount of HRP-conjugated anti-rabbit IgG 500X concentrate 500-fold with 1X Assay Diluent. Pipette up and down to mix gently.

Δ Note: Do not store and reuse after dilution.

9.8 100X HRP-Streptavidin Conjugate

Briefly spin the vial before use. Dilute the required amount of100X HRP-Streptavidin Conjugate 100-fold with 1X Assay Diluent. Pipette up and down to mix gently.

Δ Note: Do not store and reuse after dilution.

9.9 TMB One-Step Substrate Reagent

Ready to use as supplied.

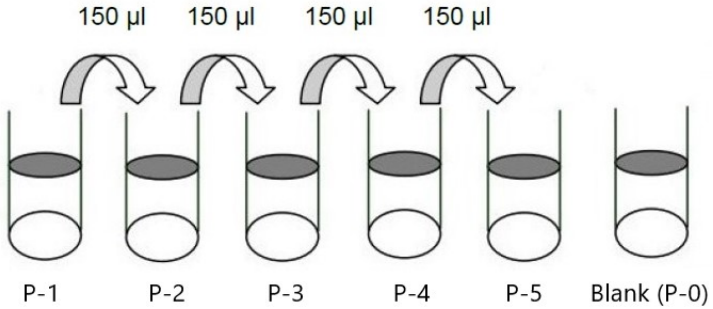
9.10 Stop Solution

Ready to use as supplied.

9.11 Positive Control

Briefly spin the Positive Control vial. Add 400 µl 1X Assay Diluent into Item K vial to prepare 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 centrifuge of the positive control vial, and then pipetting the supernate only for the assay. Pipette 300 µl 1X Assay Diluent into each tube. Use the Positive Control (P-1) to produce a dilution series (shown below). Mix each tube thoroughly before the next transfer. 1x Assay Diluent serves as the background control (P-0).

Δ Note: Store for up to 1 week at -80°C.



1X Assay Diluent:	400 µl	300 µl	300 µl	300 µl	300 µl	300 µl

10. Sample Preparation

10.1 Cell Lysates:

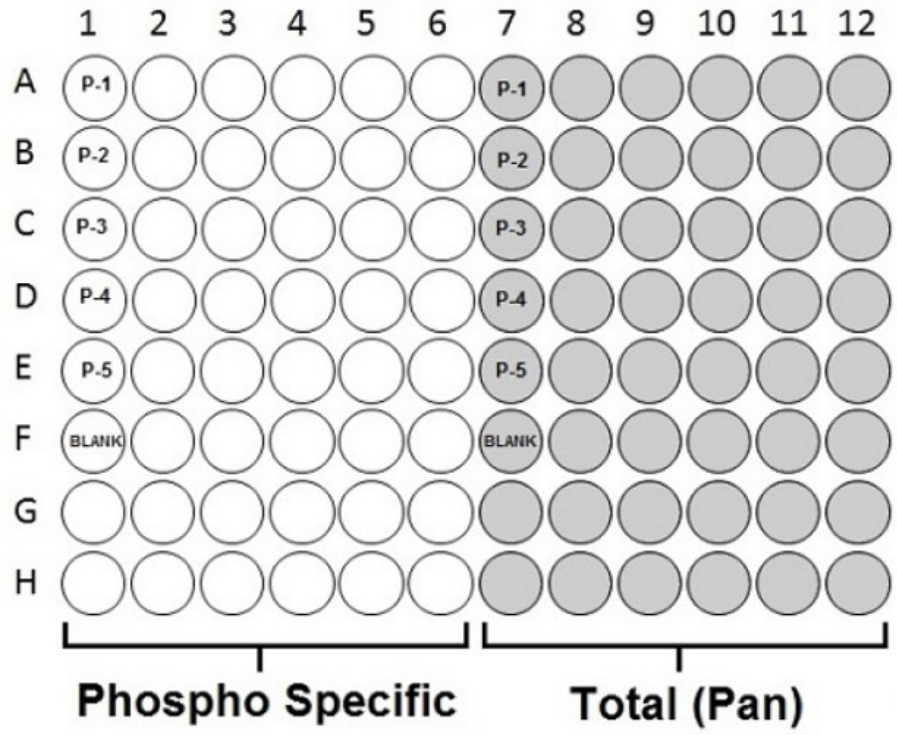
Rinse cells with PBS, making sure to remove any remaining PBS before adding the Cell Lysis Buffer. Solubilize cells at 4 x 10^7 cells/mL in 1X Cell Lysis Buffer (we recommend adding protease and phosphatase inhibitors to Cell Lysis Buffer prior to sample preparation). Pipette up and down to resuspend 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 supernates into a clean test tube. Lysates should be used immediately or aliquoted and stored at –80°C. Avoid repeated freeze-thaw cycles. Thawed lysates should be kept on ice prior to use.

For the initial experiment, we recommend a serial dilution, such as 5-fold to 100-fold dilution for your cell lysates with 1X Assay Diluent before use.

Δ 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.

11. Plate Preparation

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused well plate strips should be returned to the pouch containing desiccant pack. The pack should be resealed and stored at -20°C.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).



12. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature (18 - 25°C) prior to use.
- We recommend that you assay all standards, controls and samples in duplicate. It is also recommended to run the positive controls in singlet for each of the pan and phospho-specific antibodies.

12.1 Prepare the plate as shown on the plate layout in section 11. Label removable 8-well strips as appropriate for your experiment.

12.2 Add 100 µl of each sample or positive control into appropriate wells. Cover the wells and incubate for 2.5 hours at room temperature or overnight at 4°C with gentle shaking.

12.3 Discard the solution and wash 4 times with 1X Wash Buffer. Wash by filling each well with 300 µl 1X Wash Buffer using a multi-channel 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

12.4 Add 100 µl of prepared 1X rabbit anti-phospho-STAT1 (Tyr701) antibody into the wells designated to detect phosphorylated protein. Add 100 µl of prepared 1X biotinylated anti-STAT1 antibody into the remaining wells to detect pan STAT1 protein.

12.5 Incubate for 1 hour at room temperature with shaking.

12.6 Discard the solution. Repeat the wash as in step 12.3.

12.7 Add 100 µl of prepared 1X HRP-conjugated anti-rabbit IgG solution to the wells corresponding with rabbit anti-phospho-STAT1 (Tyr701) in order to detect phosphorylated protein. To the remaining wells (corresponding with biotinylated anti-STAT1), add 100 µl of prepared HRP-Streptavidin solution in order to detect pan STAT1 protein. Incubate for 1 hour at room temperature.

12.8 Discard the solution. Repeat the wash as in step 12.3.

12.9 Add 100 µl of TMB One-Step Substrate Reagent to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.

12.10 Add 50 µL of Stop Solution to each well.

12.11 Read the absorbance on a microplate reader at a wavelength of 450 nm immediately.

12.12 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.

13. Typical Data

Typical standard curve – data provided **for demonstration purposes only.** A new standard curve must be generated for each assay performed.

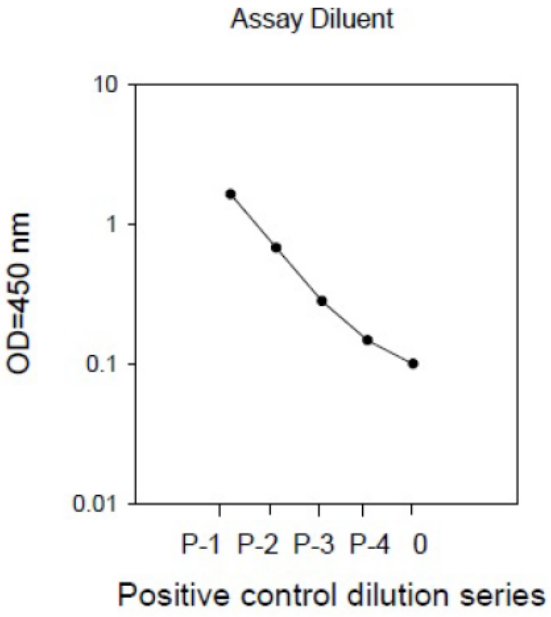


Figure 1. Example of typical standard curve. A431 cells were treated with recombinant human EGF at 37°C for 20 min. Solubilize cells at 4 x 107 cells/ml in Cell Lysis Buffer. Serial dilutions of lysates were analyzed in this ELISA.

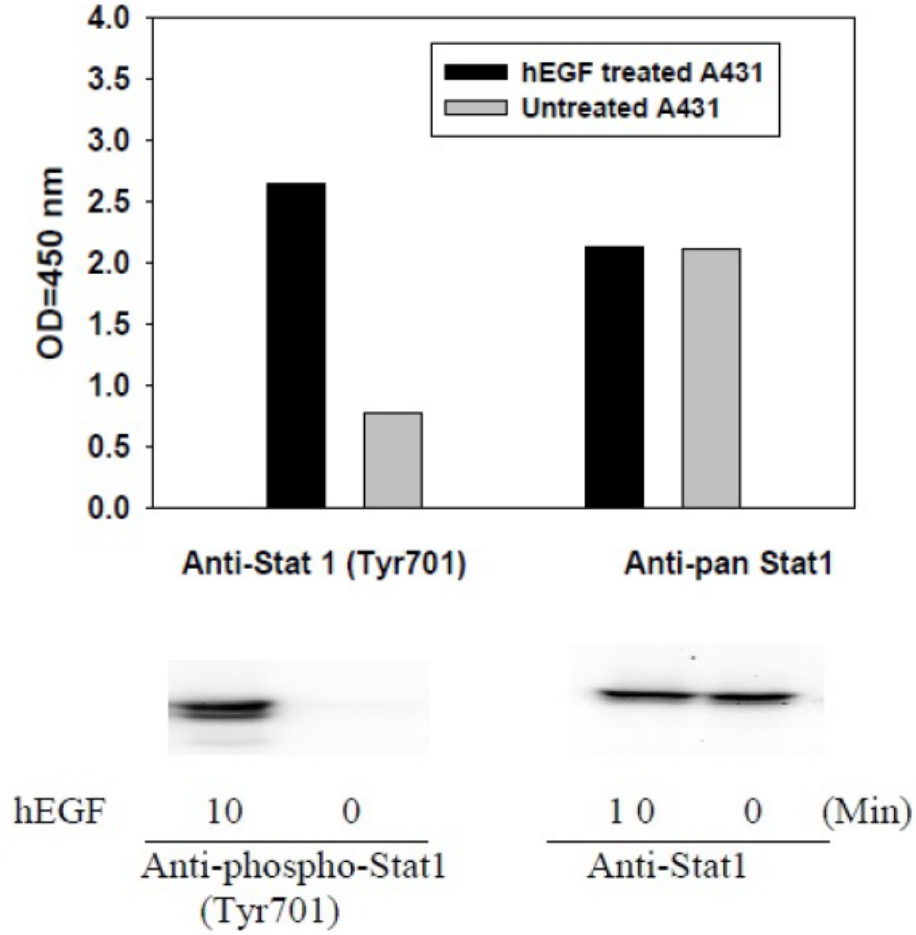


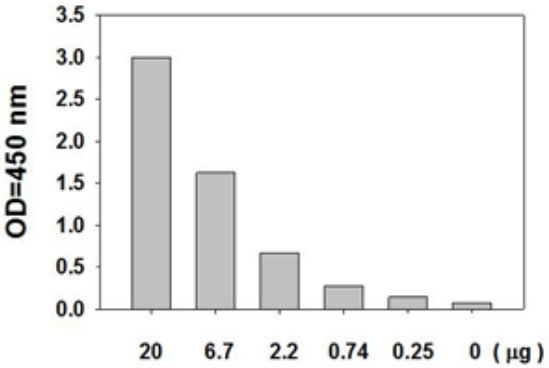
Figure 2. A431 cells were treated or untreated with 100 ng/ml recombinant human EGF for 10 min. Cell lysates were analyzed using this phospho ELISA and Western Blot.

14. Typical Sample Values

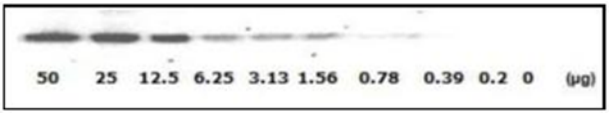
SENSITIVITY –

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 (Tyr701).

ELISA



Western Blot



15. Troubleshooting

Problem	Cause	Solution
Low signal in samples	Sample concentration is too low	Increase sample concentration
	Improper preparation of detection antibody	Briefly spin down vials before opening. Dissolve the powder thoroughly.
	Too brief incubation times	Enure sufficient incubation time; step 12.2 may be done overnight.
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
High signal in samples	Sample concentration is too high	Reduce sample concentration
Large CV	Inaccurate pipetting	Check pipettes
	Air bubbles in wells	Remove bubbles in wells
High background	Plate is insufficiently washed	Review the manual for proper wash. If using a plate washer, ensure that all ports are unobstructed.
	Contaminated wash buffer	Make fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store your positive control at <-70°C after reconstitution, others at 4°C. Keep substrate solution protected from light.
	Stop Solution	Add stop solution to each well before reading plate.
	Improper primary or secondary antibody dilution	Ensure correct dilution.

16. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)