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Product Information

Phospho-Stat3 (pTyr^{705}) ELISA Kit
for detection of phospho-stat3 (pTyr^{705})
in human, mouse, or rat cell and tissue lysates

Catalog Number **RAB0446**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

The Phospho-Stat3 (pTyr^{705}) ELISA Kit is a very rapid, convenient, and sensitive assay kit that can monitor the activation or function of important biological pathways in human, mouse, and rat cell lysates. By determining phosphorylated STAT3 protein in an experimental model system, one can verify pathway activation in 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, mouse, and rat phospho-STAT3. An anti-pan STAT3 antibody has been coated onto a 96 well plate. Samples are pipetted into the wells and STAT3 present in a sample is bound to the wells by the immobilized antibody. The wells are washed and rabbit anti-phospho-Stat3 (pTyr^{705}) antibody is used to detect phosphorylated STAT3. After washing away unbound antibody, HRP conjugated anti-rabbit IgG 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 phosphor-Stat3 (pTyr^{705}) bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

Components

1. Capture Antibody-Coated Microplate (Item A) - RABSY705A: 96 wells (12 strips × 8 wells) coated with anti-pan-STAT3.
2. Phospho-ELISA Lyophilized Positive Control Sample for Phospho-Stat3 (pTyr^{705}) (Item K) - RABSY705K: 1 vial of lyophilized powder from A431 cell lysate.
3. Anti-phospho-Stat3 (pTyr^{705}) (Item C2) - RABS705C1: 2 vials of rabbit anti-phospho-Stat3 (pTyr^{705}) (each vial is enough to assay half a microplate).
4. 20× Wash Buffer Concentrate (Item B) - RABWASH5: 25 mL of 20× concentrated solution.
5. HRP-conjugated Anti-Rabbit IgG Concentrate (Item D1) - RABHRP4: 1 vial (25 µL) of 2000× HRP-conjugated anti-rabbit IgG concentrate.
6. TMB One-Step Substrate Reagent (Item H) - RABTMB4: 12 mL of 3,3′,5,5′-tetramethylbenzidine (TMB) in buffer solution.
7. Phosphorylation ELISA Stop Solution (Item I) – RABSTOP3: 8 mL of 0.2 M sulfuric acid.
8. 5× Assay Diluent (Item E) - RABDIL11: 15 mL of 5× concentrated buffer. For diluting cell lysate samples, detection antibody (Item C2), and HRP-conjugated anti-rabbit IgG concentrate.
9. 2× Cell Lysate Buffer (Item J) - RABCLB1: 10 mL of 2× cell lysis buffer (does not include protease and phosphatase inhibitors).

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. Distilled or deionized water
8. Tubes to prepare sample dilutions
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

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 cells with PBS, making sure to remove any remaining PBS before adding the Cell Lysate Buffer. Solubilize cells at 4 × 10^7 cells/mL in 1× Cell Lysate Buffer. 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 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, a serial dilution, such as a 5-fold and 50-fold dilution of the cell lysates with prepared Assay Diluent (Item E) (see Preparation, step 2) is recommended 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

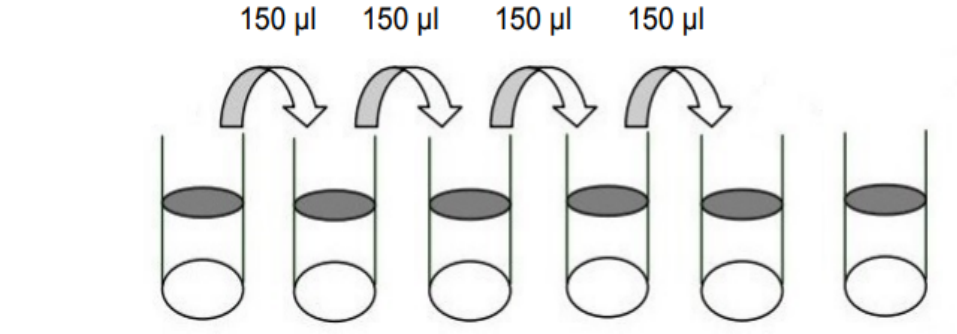
1. Bring all reagents and samples to room temperature (18–25 °C) before use.

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

3. Preparation of Positive Control – Briefly spin the Positive Control Vial (Item K). Add 500 µL of prepared 1× Assay Diluent (Item E) 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 centrifuge of the positive control vial, and then pipetting the supernatant only for the assay. Pipette 300 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).

4. 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.

Figure 1.
Dilution Series for Positive Control



	P-1	P-2	P-3	P-4	P-5		Blank (P-0)
Diluent volume	Item K + 500 µl	300 µl	300 µl	300 µl	300 µl		300 µl

5. Preparation of rabbit anti-phospho-Stat3 (pTyr^{705}) – Briefly spin the vial of rabbit anti-phospho-Stat3 (pTyr^{705}) (Item C2). Add 100 µL of 1× 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 –70 °C for one month). The concentrate should then be diluted 55-fold with 1× Assay Diluent and used in the Procedure, step 4.

6. Preparation of HRP-conjugated anti-rabbit IgG – Briefly spin the vial of HRP-conjugated anti-rabbit IgG concentrate (Item D1) before use. HRP-conjugated anti-rabbit IgG should be diluted 2000-fold with 1× Assay Diluent and used in step 7 of the Assay Procedure.

   For example: Briefly spin the vial (Item D1) and pipette up and down to mix gently. Add 5 µL of HRP-conjugated anti-rabbit IgG concentrate into a tube with 10 ml of 1× Assay Diluent to prepare a 2000-fold diluted HRP-conjugated anti-rabbit IgG solution.

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 –80 °C (–80 °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 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. Label removable 8 well strips as appropriate for the experiment.

3. 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 over night at 4 °C with 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 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.

5. Add 100 µL of prepared 1× rabbit anti-phospho-Stat3 (pTyr^{705}) (Preparation, step 5) to appropriate wells. Incubate for 1 hour at room temperature with shaking.

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

7. Add 100 µL of prepared HRP-conjugated anti-rabbit IgG solution to corresponding well. Incubate for 1 hour at room temperature with 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 shaking.

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

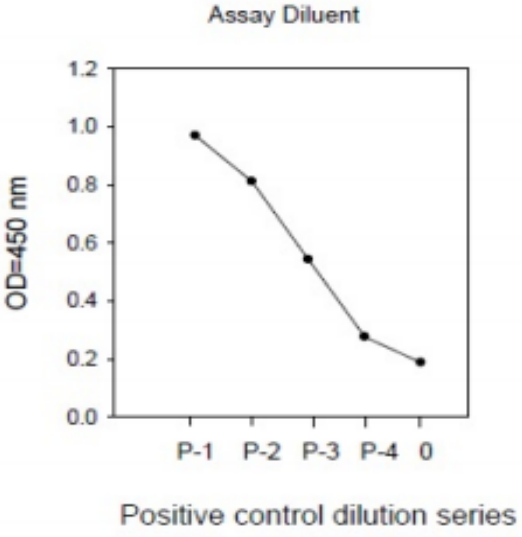
Results

Typical Data

Calculate the mean absorbance for each set of duplicate positive controls and samples, and then subtract the average zero (blank) optical density.

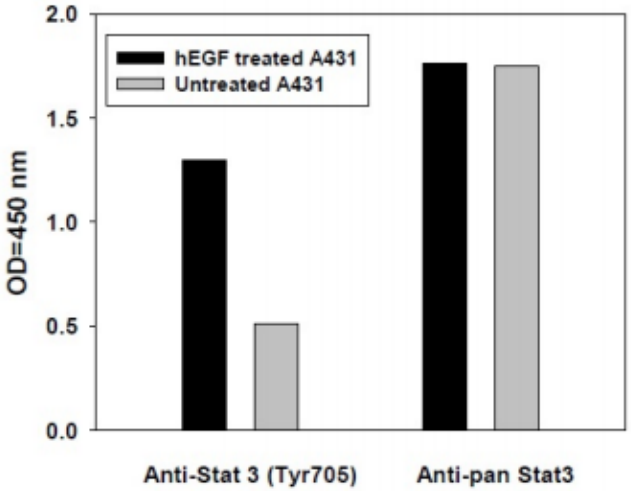
Positive Control

A431 cells were treated with recombinant human EGF at 37 °C for 20 minutes. Cells were solubilzed at 4×10^7 cells/mL in Cell Lysate Buffer. Serial dilutions of lysates were analyzed with this ELISA.

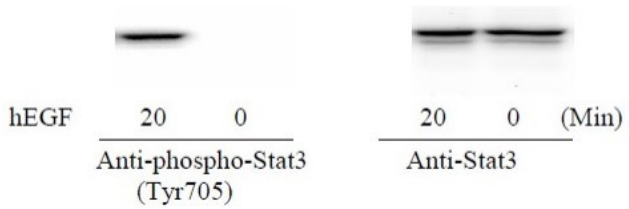


Recombinant Human EGF Stimulation of A431 Cell Lines:

ELISA



Western blot

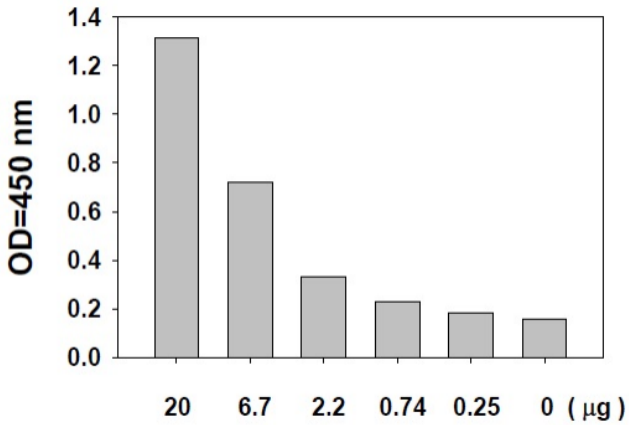


Product Profile

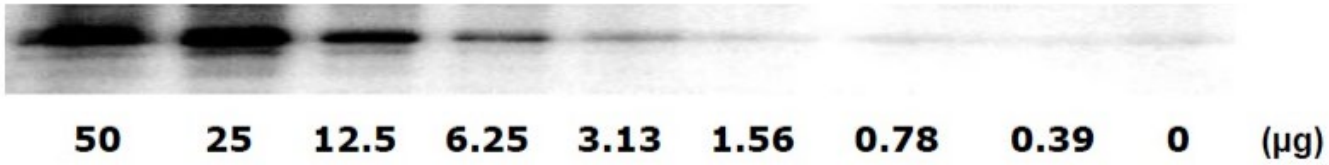
Sensitivity

The A431 cells were treated with 100 ng/mL recombinant human EGF for 20 minutes to induce phosphorylation of Stat3. Serial dilutions of lysates were analyzed with this ELISA and by Western blot. Immunoblots were incubated with antiphospho-Stat3 ($pTyr^{705}$).

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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