Signosis
Innovative Plate Assay Solutions

Stat3/Stat1 ELISA Kit

Catalog Number TE-0025 **(For Research Use Only)**

Introduction

Stat3 (signal transducer and activator of transcription 3) promotes cell survival/proliferation, motility and immune tolerance and is considered as an oncogene. When signaled by cytokines and growth factors, such as oncostate, the activated Stat3 is translocated into the nucleus, where the gene expression is regulated by binding to the DNA recognition site.

STAT1 exerts a wide spectrum of functions on both tumor cells and the immune system. Stat1 is usually considered as a tumor suppressor by enhancing inflammation and triggering anti-proliferative and pro-apoptotic responses in tumor cells. Upon activated by cytokines, such as INFgamma, stat1 forms homodimers or heterodimers with Stat3 or Stat2 and binds to the response element on the promoter region of target genes.

While STAT3 promotes cell survival/proliferation, motility and immune tolerance and is considered as an oncogene, STAT1 enhances inflammation and innate and adaptive immunity, triggering in most instances anti-proliferative and pro-apoptotic responses in tumor cells. Despite being activated by common cytokines and growth factor receptor pathways, their activation is reciprocally regulated to redirect and balance cytokine/growth factor signals from proliferative to apoptotic, or from inflammatory to anti-inflammatory. Signosis has developed Stat3/Stat1 ELISA kit to distinguish the activation of Stat3 and Stat1.

Principle of the assay

Stat3/Stat1 ELISA kit is high sensitive and specific assay with a simple and optimized procedure. The 96-well (8X6 strip for Stat3 and 8X6 strip for Stat1) clear plate is pre-immobilized with the Stat3/Stat1 consensus sequencing oligo. The activated Stat3/Stat1 in nuclear extract or the whole cell lysate is added in the well and binds to the oligo. The activated Stat3/Stat1 is detected with a specific antibody against Stat3/Stat1 subunit and a HRP conjugated secondary antibody. The assay utilizes colorimetric detection method, which can be easily measured by spectrophotometry.

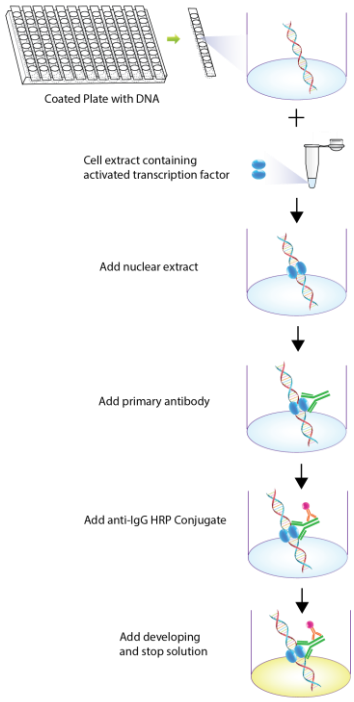


Diagram of TF ELISA

Materials provided with the kit

- 8x12 96-well microplate coated with Stat3/Stat1 consensus oligo (4°C)
- Antibody against Stat3 (4°C)
- Antibody against Stat1 (4°C)
- HRP conjugate secondary antibody (4°C)
- 2X TF binding buffer (-20°C)
- 1X Nuclear extract dilution buffer (-20°C)
- Stat3 Positive control (-20°C)
- Stat1 Positive control (-20°C)
- 1X Diluent buffer (4°C)
- 5X Assay wash buffer (4°C)
- Substrate (4°C)
- Stop Solution (4°C)

Material required but not provided

- Microplate reader capable of measuring absorbance at 450 nm
- Deionized or distilled water.

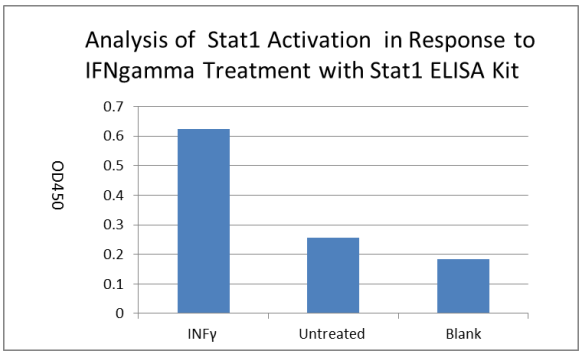
Reagent preparation before starting experiment

- Dilute the 5x Assay wash buffer to 1x buffer
  40ml 5x Assay wash buffer
  160ml ddH2O
- Dilute 250 times of antibody against Stat3 with 1X Diluent buffer before use.
- Dilute 250 times of antibody against Stat1 with 1X Diluent buffer before use.
- Dilute 1000 times of HRP conjugate secondary antibody with 1X Diluent buffer before use.

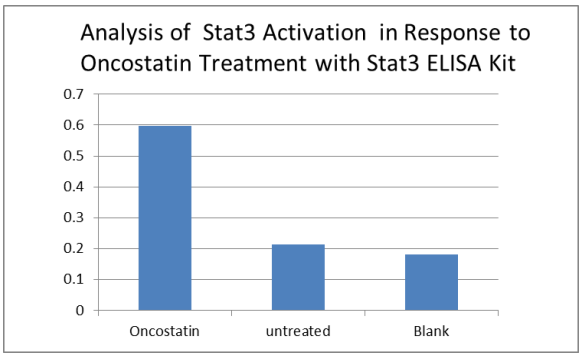
Assay procedure

1. Calculate the number of samples to decide how many strips need to be used.
2. Make TF binding mix
   25ul 2X TF binding buffer
   X Nuclear extract (2-10ug)
   X Nuclear extract dilution buffer
   Total 50ul
   For positive control, use 25ul of positive control without adding unclear extract dilution buffer.
3. Add the mix on a well and incubate for 1 hour with gently shaking at room temperature.
4. Discard the contents and wash by adding 200µl of 1X Assay wash buffer. Repeat the process three times for a total of three washes. Complete removal of liquid at each wash. After the last wash, remove any remaining liquid by inverting the plate against clean paper towels.
5. Add 100µl of diluted antibody against Stat3 or Stat1 to the corresponding well and incubate for 1 hour at room temperature with gentle shaking.
6. Repeat the aspiration/wash as in step 4.
7. Add 100 µl of diluted HRP conjugate secondary antibody to each well and incubate for 45 min at room temperature with gentle shaking.
8. Repeat the aspiration/wash as in step 4.
9. Add 100µl of substrate to each well and incubate for 15-30 minutes.
10. Add 50µl of stop solution to each well. The color in the wells should change from blue to yellow.
11. Determine the optical density of each well with a microplate reader at 450 nm within 30 minutes.

Example of standard curve



Stat1 ELISA analysis. Hela cells were treated with and without 10ng/ml IFNgamma for 1 hours, and the nuclear extracts were prepared and subjected to Stat1 ELISA kit



Stat3 ELISA analysis. Hela cells were treated with and without 10ng/ml oncostatin for 1 hours, and the nuclear extracts were prepared and subjected to Stat3 ELISA kit.