**Signosis**
Innovative Plate Assay Solutions

# STAT1 ELISA Kit (Colorimetric)

**Catalog Number TE-0023** **(For Research Use Only)**

## Introduction

STAT1 (Signal Transducer and Activator of Transcription 1) plays an important role in immune regulation and tumor suppression. Upon activation by cytokines such as interferon-γ (IFN-γ), STAT1 forms dimers and translocates to the nucleus, where it binds DNA response elements to regulate target gene expression. STAT1 promotes inflammation and immune responses while often inducing anti-proliferative and pro-apoptotic effects in tumor cells. Signosis has developed the STAT1 ELISA kit for the analysis of the STAT1 pathway and to facilitate studying activation of different STAT1-related pathways.

## Principle of the assay

The STAT1 ELISA kit is a highly sensitive and specific assay with a simple and optimized procedure. The 96-well (8X12 strip) clear plate is pre-immobilized with the STAT1 consensus sequencing oligo. When your nuclear extract or whole cell lysate is added to the well, activated STAT1 binds to the oligo. The activated STAT1 is detected with a specific antibody against an STAT1 subunit and a HRP conjugated secondary antibody. The assay utilizes colorimetric detection, which can be easily measured by spectrophotometry.

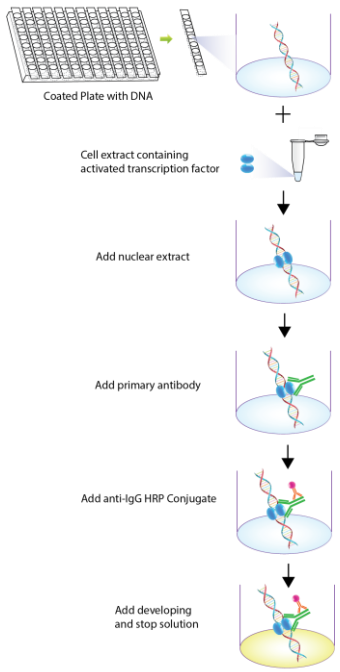


Diagram of TF ELISA

## Materials provided with the kit

- 8x12 96-well microplate coated with STAT1 consensus oligo (4ºC).
- 100X STAT1 Primary antibody (4ºC).
- HRP conjugate secondary antibody (4ºC)
- 2X Binding buffer (-20ºC).
- STAT1 positive control, lyophilized (4ºC)
- 1X Diluent buffer (4ºC)
- 10X Assay wash buffer (4ºC)
- Substrate (4ºC)
- Stop Solution (4ºC)

## 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 10X Assay wash buffer with ddH2O to 1x wash buffer.
- Reconstitute STAT1 positive control in 100ul 1X Binding buffer (2X Binding buffer diluted with ddH2O). Store reconstituted STAT1 positive control at -80°C.
- Dilute 100X STAT1 Primary antibody 1:100 with 1X Diluent buffer before use.
- Avoid contact of Substrate and Stop Solution with any metal surfaces.
- Important Note: Before beginning your experiment, quickly take 50µL of the Substrate and check to make sure it's clear. **If its color has changed to dark blue, do not begin the experiment and contact us immediately.**

## Assay procedure

**Step 1: Binding of STAT1 to its Consensus Sequence**

1. Calculate the number of samples to decide how many strips need to be used.

2. Make Binding mix for samples/control

   50ul 2X Binding buffer
   X Nuclear extract (10-20ug)
   X ul ddH2O
   Total 100ul

   For the positive control, add 5ul of reconstituted positive control and 45ul ddH2O with 50ul 2X Binding buffer.
   For the negative control, add 50ul of ddH2O with 50ul 2X Binding buffer.

   Add the samples/controls in the wells and incubate for 2 hours at room temperature on a shaker.

3. Discard the contents and wash by adding 200µl of 1X Assay wash buffer. Repeat the process for a total of three washes. Completely remove the liquid at each wash. After the last wash, remove any remaining liquid by inverting the plate against clean paper towels.

**Step 2: Binding of Primary Antibody**

4. Add 100 µl of diluted STAT1 Primary antibody to each well and incubate for 2 hours at room temperature on a shaker.
5. Repeat the aspiration/wash steps in Step 1. No,3

**Step 3: Binding of Secondary Antibody**

6. Add 100 µl of the HRP conjugate secondary antibody to each well and incubate for 90 minutes at room temperature on a shaker.
7. Repeat the aspiration/wash steps in Step 1. No,3

**Step 4: Colorimetric Reaction**

8. Before adding substrate, check to make sure it is clear. **If the substrate is already blue, please leave the wash buffer in the wells, seal and store the plate at 4°C, and contact us immediately.**
9. Add 100 µl of substrate to each well and incubate for 10-30 minutes away from light. Check the plate for the development of blue color in the wells.
10. When color development is sufficient, add 100µl 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 immediately.