Signosis
Innovative Plate Assay Solutions

SATB1 ELISA Kit (Colorimetric)

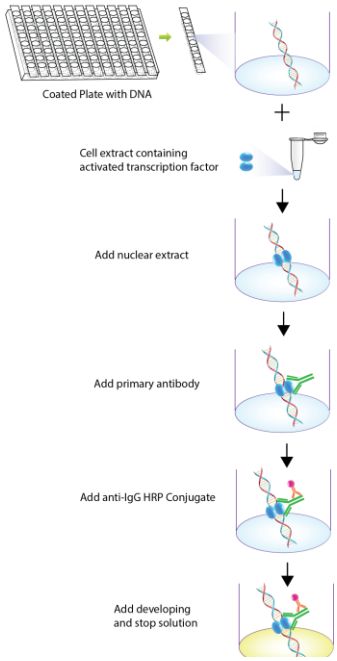
Catalog Number TE-0008 **(For Research Use Only)**

Introduction

SATB1 is a nuclear protein which plays a crucial role in metastasis of breast cancer. It promotes tumor growth and metastasis by changing the expression of hundreds of genes, affecting cell adhesion, cell signaling, cell-cycle regulation, and other functions, including the epidermal growth factor gene Her2. SATB1 is not expressed in all cells. Only the metastatic cells express SATB1, and the most aggressive breast cancer cells show the highest levels of the protein. When SATB1 is detected in a breast tumor, the cancer is highly likely to progress or recur. Studies with human primary breast cancer tissue samples indicated that the highest levels of SATB1 were in samples from patients whose survival times had been shortest; patients whose tumor samples had no SATB1 expression generally had longer survival times. In addition, SATB1's ability to regulate gene expression was identified as critical to T-cell development. Studies revealed that SATB1 interacts with HDAC1 or PCAF, and is regulated by phosphorylation and cleaved by Caspase 6. The expression is high in Jurkat and low in HeLa cells. Signosis offers SATB1 ELISA kit to facilitate the measurement of this important protein in cancer metastasis..

Principle of the assay

SATB1 kit is high sensitive and specific assay with a simple and optimized procedure. The 96-well (8X12 strip) clear plate is pre-immobilized with the SATB1 consensus sequencing oligo. The activated SATB1 in nuclear extract or the whole cell lysate is added in the well and binds to the oligo. The activated SATB1 is detected with a specific antibody against SATB1 subunit and a HRP conjugated secondary antibody. The assay utilizes colorimetric detection method, which can be easily measured by spectrophotometry.



Materials provided with the kit

- 8x12 96-well strip plate coated with SATB1 consensus oligo (4ºC).
- Mouse anti-SATB1 antibodies (-20ºC).
- Anti-mouse HRP conjugate secondary antibody (4ºC)
- 2X TF binding buffer (-20ºC).
- 1X Nuclear extract dilution buffer (-20ºC).
- Positive control (-80ºC)
- 1X Diluent buffer (4ºC)
- 5X 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 5x Assay wash buffer to 1x buffer
  40ml 5x Assay wash buffer
  160ml ddH2O
- Dilute 200 times of antibody against mouse anti-SATB1 with 1X Diluent buffer before use.
- Dilute 500 times of anti-mouse 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
   Add 30ul 2X TF binding buffer on each assay well, then add
   X Nuclear extract (2-10ug)
   X Nuclear extract dilution buffer
   To make total 60ul/per well
   Mix well by pipetting up and down.

   Note: For the positive control, add 30ul positive control nuclear extract provided.
   For blank, add 30ul ddH2O or Nuclear extract dilution buffer
3. Incubate at room temperature for 1 hour with gently shaking.
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. Completely remove liquid at each wash. After the last wash, remove any remaining liquid by inverting the plate against clean paper towels.
5. Add 60µl of diluted antibody against SATB1 to each well and incubate for 2 hours at room temperature with gentle shaking, or 4 ^{o}C overnight with sealing without shaking.
6. Repeat the aspiration/wash as in step 4.
7. Add 60 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 60µl of substrate to each well and incubate for 10-20 minutes, or longer until the blue color is developed.
10. Add 30µl 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 450nm within 30 minutes.