



Human SATB1 ELISA

Catalog Number EA-2001

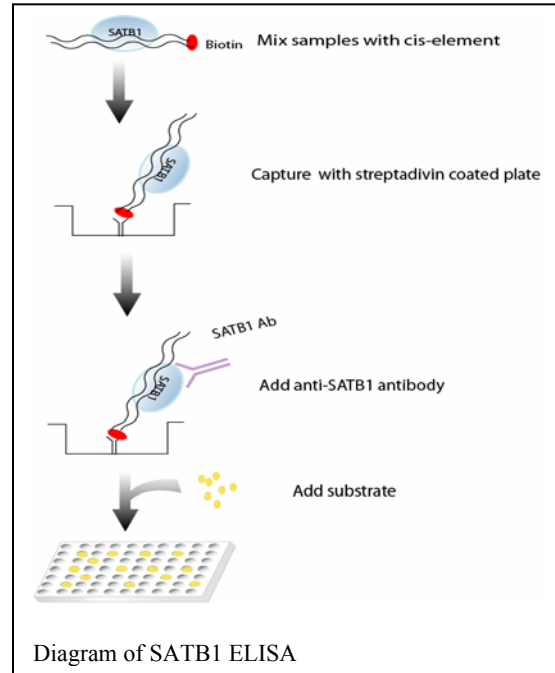
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Introduction

SATB1 is a nuclear protein which plays a crucial role in metastasis of breast cancer (1). 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 expressed SATB1, with the most aggressive breast cancer cells showing 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 for which clinical follow-up studies 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, is regulated by phosphorylation and cleaved by Caspase 6. It expresses high in Jurkat and low in HeLa cells. Signosis offers SATB1 ELISA kit to facilitate the measurement of the important protein in cancer metastasis.

Principle of the assay

SATB1 ELISA is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay utilizes biotin labeled DNA binding sequence of SATB1 for immobilization of SATB1/DNA complex onto the streptavidin-coated microtiter wells, which is detected by an anti-SATB1 antibody and anti-IgG conjugated to horseradish peroxidase (HRP). After incubation, the wells are washed to remove unbound proteins. A HRP substrate, TMB, is added to result in the development of a blue color. The color development is then stopped with the addition of Stop Solution changing the color to yellow. The concentration of SATB1 is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.



Materials provided with the kit

- Assay strips 12 X 8 wells coated with streptavidin (4°C).
- 2X Binding buffer with SATB probe (-20°C)
- Mouse anti-human SATB1 antibody (-20°C).
- Anti-mouse IgG conjugated HRP conjugate (4°C)
- Jurkat nuclear extract control (-20°C)
- Nuclear extract dilution buffer (-20°C)
- 1X Assay blocking buffer (4°C)
- 5X Assay wash buffer (RT)
- 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 2X Binding buffer with ddH₂O at 1:1 to 1X Binding buffer
- Dilute the 5X Assay wash buffer to 1X buffer
40ml 5X Assay wash buffer
160ml ddH₂O
- Dilute 500 times of mouse anti SATB1 monoclonal antibody with 1X Assay blocking buffer
- Dilute 500 times of anti-mouse IgG HRP conjugate with 1X Assay blocking buffer.
- Dilute nuclear extract or whole cell lysate to 0.5ug-2.0µg/µl with Nuclear extract dilution buffer.

Assay procedure

1. Calculate the desired samples, and designate the wells of assay strips to be used.
2. Use PCR tubes to mix the following components for each reaction: add 40ul of 1X Binding buffer to each tube and mix with 10µl of 0.5ug-2.0µg/µl nuclear extract or whole cell lysate. Use 10µl of Jurkat nuclear extract control as a positive control.
3. Incubate for 30 minutes at room temperature.
4. Transfer 45 µl of each reaction mix to individual wells of the assay strip and incubate for 1 hour at room temperature with gentle shaking.
5. Aspirate each well 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 by inverting the plate against clean paper towels forcibly.
6. Add 100µl of diluted anti SATB antibody to each well and incubate for 1 hour at room temperature with gentle shaking.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µl of diluted anti mouse IgG-HRP conjugate to each well and incubate for 30 min at room temperature with gentle shaking.
9. Repeat the aspiration/wash as in step 5.
10. Add 100µl of substrate to each well, and incubate for 5-15 minutes.
11. Add 50µl of Stop solution to each well. The color in the wells should change from blue to yellow.
12. Determine the optical density of each well with a microplate reader at 450 nm within 30 minutes.

References

1. Han HJ, Russo J, Kohwi Y, Kohwi-Shigematsu T. 2008. SATB1 reprogrammes gene expression to promote breast tumour growth and metastasis. *Nature*. 452:187-93.
2. Kumar PP, Purbey P, Sinha C, Notani D, Limaye A, Jayani R, Galande S. 2006 Phosphorylation of SATB1, a Global Gene Regulator, Acts as a Molecular Switch Regulating Its Transcriptional Activity In Vivo. *Molecular Cell*, 22:231 – 243.
3. Galande S, Dickinson LA, Mian S, Sikorska S, and Kohwi-Shigematsu T, 2001, SATB1 Cleavage by Caspase 6 Disrupts PDZ Domain-Mediated Dimerization, Causing Detachment from Chromatin Early in T-Cell Apoptosis. *Mol. Cell. Biol.* 21:5591–5604.

Example of SATB1 ELISA

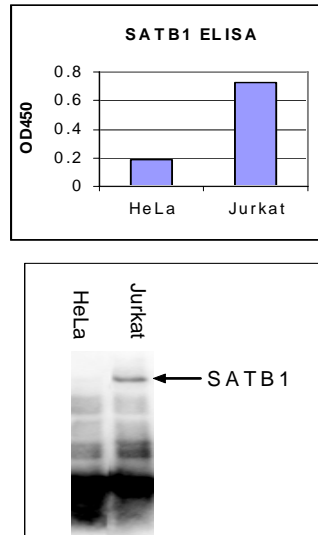


Figure: ELISA (top) and gel shift analyses (bottom) of SATB1 in Jurkat and HeLa cells.