**Signosis**
Innovative Plate Assay Solutions

# OCT4 ELISA Kit (Colorimetric)

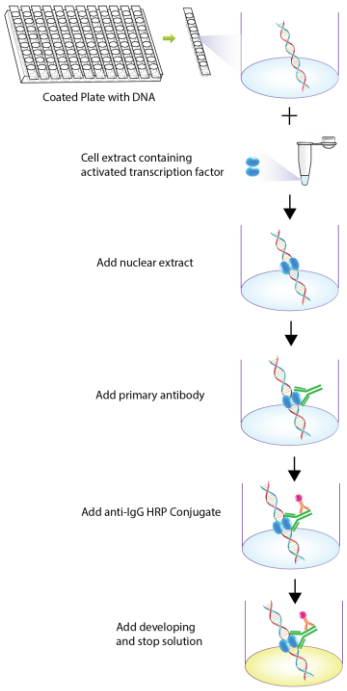
**Catalog Number TE-0031** **(For Research Use Only)**

## Introduction

Two key transcription factors, Oct4 and Sox2, have been identified that are crucial for maintenance of the pluripotent state of ESCs. Octamer 3/4 (Oct 3/4), a member of the POU family, is considered to be an important stem cell marker and essential transcription factor during human embryogenesis. Sox2 is a member of the Sox (SRY-Related HMG box) gene family that encodes transcription factors with a single HMG DNA-binding domain. Sox2 controls neural progenitor cells by prohibiting their ability to differentiate. In recent years, there have also been reports about the presence of Oct 3/4 and Sox2 in differentiated benign and malignant human cells. Oct4 and Sox2 showed cooperative binding to the Sox-Oct motifs on some target promoter regions. In addition, the transcription factor Nanog, an established regulator of pluripotency, is transcriptionally regulated directly by Oct4 and Sox2. Signosis has developed an ELISA kit to facilitate the study of Oct4 and Sox2 pathway.

## Principle of the assay

Oct4/Sox2 ELISA kit is high sensitive and specific assay with a simple and optimized procedure. The 96-well (12 Strips) clear plate is pre-immobilized with Oct4 or Sox2 consensus sequencing oligo. The activated Oct4 or Sox2 in nuclear extract or the whole cell lysate is added in the well and binds to the oligo. The activated Oct4 or Sox2 is detected with a specific antibody against Oct4 or Sox2 and a HRP conjugated secondary antibody. The assay utilizes colorimetric detection method, which can be easily measured by spectrophotometry.




## Materials provided with the kit

- 96-well microplate coated with Oct4 consensus oligo (4ºC).
- Antibody against Oct4 (4ºC).
- HRP conjugate secondary antibody (4ºC)
- 2X TF binding buffer (-20ºC).
- Nuclear extract dilution buffer (-20ºC).
- Oct4 positive control (-80ºC)
- Negative control for Oct4 (-80°C)
- 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 **100** times of antibody against Oct4 with 1X Diluent buffer before use.
- Dilute **500** 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 the positive control, add 25ul positive control nuclear extract provided.
   For the negative control or blank, add 25ul negative control provided.
3. Add the mix on a well and incubate at room temperature for 1-2 hours (or overnight at 4°C) without shaking.
4. Invert and 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 50µl of diluted antibody against Oct4 to each well and incubate for 1 hour at room temperature with gentle shaking.
6. Repeat the aspiration/wash as in step 4.
7. Add 50 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 50µl of substrate to each well and incubate for 10-15 minutes.
10. Add 25µl 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.