

Oct4 (Human) Transcription Factor Activity Assay Kit

07/17

(Catalog # E4336-100, 100 assays, Store at -20°C)

I. Introduction:

Pluripotency and differentiation of embryonic stem (ES) cells is controlled by regulatory genes, which are often transcriptional regulators. The roles of these regulators are to repress or activate patterns of gene expression for normal pluripotent cell development and maintenance, and to mediate permanent phenotypic change during stem cell differentiation. Oct4 is one of these important transcription factors specifically expressed in cells with pluripotent identity. For example, a study with Oct4-null mouse ES cells that differentiated into trophectoderm, the lineage that supplies trophoblast cells for the developing placenta, defined the effects of Oct4 on ES cell self-renewal¹. Oct4 belongs to the Octamer class of transcription factors because of recognition of an 8-bp consensus ATGCAAAT DNA sequence. Together with Pit and Unc proteins, Oct proteins define the POU (Pit, Oct and Unc) domain that interact with DNA by binding to the 8-bp sequence. Oct4 contains two DNA-binding domains: the 75-amino-acid POU-specific domain (POU_S) and the 60-amino-acid carboxy-terminal POU homeodomain (POU_{HD}). Accurate monitoring of the level of activated Oct4 in cells, tissues or animal models is required for investigating signal transduction pathways and other research applications such as drug development. Simple, speedy and high-throughput methods are required for this purpose. The BioVision Oct4 Transcription Factor-Activity Assay kit is a non-radioactive transcription factor assay with an ELISA format. It offers an easy, speedy, sensitive and high-throughput method to detect the activation of transcription factors.

Application:

This ELISA kit is used for in vitro quantitative determination of active Oct4

II. Sample Type:

Nuclear extraction, whole lysates

III. Kit Contents:

Components	E4336-100	Part No.	Storage after Preparation
Microplate	12x8 wells	E4336-100-1	4°C for one month
DNA Binding Buffer (5X)	4 ml	E4336-100-2	4°C
Positive Control	20 µl	E4336-100-3	-20°C / -80°C
Specific Competitor DNA Probe	1 vial	E4336-100-4	4°C
Non-specific Competitor DNA Probe	1 vial	E4336-100-5	4°C
Assay Reagent	200 µl	E4336-100-6	4°C
DTT (300 mM)	200 µl	E4336-100-7	4°C
Wash Buffer Concentrate (20X)	25 ml	E4336-100-8	4°C
Oct4 Primary Antibody	1 vial	E4336-100-9	4°C for 5 days
HRP-conjugated Secondary Antibody	1 vial	E4336-100-10	4°C for 5 days
Antibody Diluent Buffer	25 ml	E4336-100-11	4°C
TMB One-Step Substrate Reagent	12 ml	E4336-100-12	4°C
Stop Solution	8 ml	E4336-100-13	4°C

IV. User Supplied Reagents and Equipment:

- Protease and Phosphatase inhibitors
- Microplate reader capable of measuring absorbance at 450 nm
- Absorbent paper
- Distilled or deionized water

V. Storage and Handling:

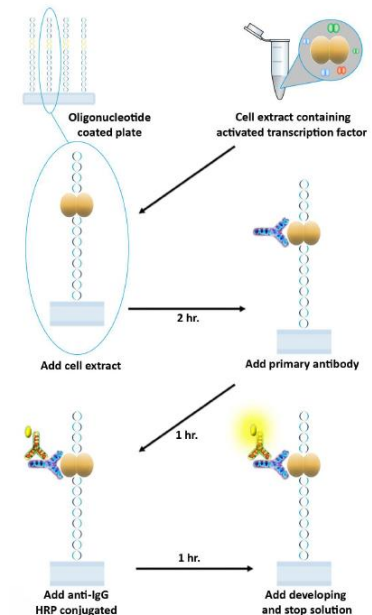
The kit can be used within one year if the whole kit is stored at -20°C upon receipt. Avoid repeated freeze-thaw cycles. Storage condition for opened reagents are listed in section IV.

VI. Reagent and Sample Preparation:

Note: Bring all reagents and samples to room temperature before use. Thaw the positive control and samples and keep them on ice before adding into wells. Please note that the amount of total protein containing the target protein to be used in this test can be optimized and must be determined by the end users.

- **Transcription factor binding reaction system:** Prepare 100 µl transcription factor binding reaction system for each well with 5 x DNA Binding Buffer, ATF-ELISA assay Reagent, DTT, Specific Competitor DNA Probe, Non-specific Competitor DNA Probe, and Positive Control or samples containing targeted proteins. Typical examples are shown in the table below.

Note: Each reaction may be prepared in a labeled microfuge tube or directly in the coated plate well. If the reaction system is prepared directly in the coated plate wells, please add the reagents sequentially as shown in the table to get the best results.





- **Primary antibody:** Briefly spin down the Oct4 Primary Antibody vial. Add 100 µl of Antibody Diluent Buffer into the vial to prepare a primary antibody concentrate. Pipette up and down to mix gently. The primary antibody concentrate should then be diluted 100-fold with the Antibody Diluent.
- **Secondary antibody:** Briefly spin down the HRP-conjugated Secondary Antibody vial before use. Add 100 µl of Antibody Diluent Buffer into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently. The detection antibody concentrate should then be diluted 100-fold with the Antibody Diluent Buffer.
- **Wash Buffer:** Dilute 25 ml of the 20x Wash Buffer Concentrate into deionized or distilled water to yield 500 ml of 1x Wash Buffer. If the Wash Buffer Concentrate (20x) contains visible crystals, warm to room temperature and mix gently until dissolved.

COMPONENT	REACTION				
	Positive control	Sample	Specific competitor	Non-Specific competitor	Blank
5x TF-Activity Assay DNA Binding Buffer	20 µl	20 µl	20 µl	20 µl	20 µl
TF-Activity Assay Reagent	1.5 µl	1.5 µl	1.5 µl	1.5 µl	1.5 µl
DTT	1 µl	1 µl	1 µl	1 µl	1 µl
Specific Competitor	-	-	10 µl	-	-
Non-specific Competitor	-	-	-	10 µl	-
Control/Sample containing proteins	5 µl	* µl	* µl	* µl	-
Total volume	bring final volume to 100µl with deionized water	bring final volume to 100µl with deionized water	bring final volume to 100µl with deionized water	bring final volume to 100µl with deionized water	bring final volume to 100µl with deionized water

VII. Assay Protocol:

Note: Bring all reagents to room temperature (18 - 25°C) before use. A positive control should be included every time to confirm correct operation of experiment, however it is not necessary to run specific competitor and non-specific competitor for each sample and every time. It is recommended that all positive control and samples be run at least in duplicate.

1. Add 100 µl of each prepared **transcription factor binding reaction system** including **positive control, specific competitor, non-specific competitor** and **samples** into appropriate wells. Cover wells and incubate for 2 hours at room temperature or overnight at 4°C with gentle shaking.
2. Discard the solution and wash 4 times by filling each well with 300 µl of 1x **Wash Buffer**. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
3. Add 100 µl of prepared **Oct4 Primary Antibody** to each well. Incubate for 1 hour at room temperature with gentle shaking.
4. Discard the solution. Repeat the wash as in step 2.
5. Add 100 µl of prepared **HRP-conjugated Secondary Antibody** to each well. Incubate for 1 hour at room temperature with gentle shaking.
6. Discard the solution. Wash as directed in step 2.
7. Add 100 µl of **TMB One-Step Substrate Reagent** to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.
8. Add 50 µl of **Stop Solution** to each well. Read at 450 nm immediately.

VIII. Typical Data:

- (Left) Transcription factor activity assay of Oct4 from nuclear extracts of P19 cells.
- (Right) Transcription factor activity assay of Oct4 from nuclear extracts of P19 cells with the specific competitor or non-specific competitor. The result shows specific binding of Oct4 to the conserved DNA binding site.

