

BACKGROUND

Oct4 (also known as Oct-3) belongs to the POU (Pit-Oct-Unc) transcription factor family. The POU family of transcription factors can activate the expression of their target genes through binding an octameric sequence motif of an AGTCAAT consensus sequence. It was shown that Oct4 is almost exclusively expressed in ES cells. During embryonic development, Oct4 is expressed initially in all blastomeres. Subsequently, its expression becomes restricted to the ICM and downregulated in the TE and the primitive endoderm. At maturity, Oct4 expression becomes confined exclusively to the developing germ cells. Targeted disruption of Oct4 in mice has produced embryos devoid of a pluripotent ICM, suggesting that Oct4 is required for maintaining pluripotency. Furthermore, quantitative analysis of Oct4 expression revealed that a high level of Oct4 expression drives ES cells towards the extra-embryonic mesoderm or endoderm lineages, while those with a low level of Oct4 become trophectodermal cells; ES cells with a normal level of Oct4 remain pluripotent. Thus, it has been proposed that Oct4 is a key regulator of stem cell pluripotency and differentiation.¹

The hallmark feature of the POU family of transcription factors is the POU domain, which consists of two structurally independent subdomains: a 75 amino acid amino-terminal POU specific (POUs) region and a 60 amino-acid carboxyl-terminal homeodomain (POUh). Both domains make specific contact with DNA through a helix-turn-helix structure and are connected by a variable linker of 15 to 56 amino-acids. The N-terminal domain (N domain) is rich in proline and acidic residues, while the C-terminal domain (C domain) is rich in proline, serine and threonine residues. The N domain has traditionally been accepted for its role in transactivation. The C domain also plays a role in transactivation. It was demonstrated that the activity of Oct4 C domain is cell type specific and is regulated through phosphorylation, whereas the N domain is not. Expression of Oct4 is regulated at the transcription level by cis-acting elements located upstream of the Oct4 gene and methylation of chromatin structure.²

Oct4 is different from many known transcription regulators that appear to function in a binary on-off manner. In some cases, Oct4 can act as a repressor of target genes whereas in other cases, it acts as an activator. Oct4 controls the pluripotency of stem cells in a quantitative fashion. It was shown that high level of Oct4 expression drives ES cells to endoderm and mesoderm lineages, while stem cells with low level of Oct4 differentiate into TE. Only a "normal level" of Oct4 can retain stem cells in a pluripotent state. In addition, Oct4 may function through other transcription factors to activate or repress target genes. It was shown that Oct4 could repress the expression of FoxA1 and FoxA2 through an

interaction with the DNA binding domain of their activator FoxD3. Since Oct4 does not bind to the promoters of FoxA1 and FoxA2, it behaves as a corepressor of these promoters. The demonstration that four transcription factors, Oct4/Sox2/Myc/Klf4, can reprogram fibroblasts into ES-like cells or induced pluripotent stem cells (iPS cells) has generated tremendous interests not only in the field of stem cell biology, but also those related fields such as developmental biology and regenerative medicine.³ Finally, many studies suggest a link between Oct4 and tumorigenesis.⁴ Like ES cells, tumor cells exhibit a unique pattern of gene expression, and thus, may be under the control of one or more master regulators like Oct4. Therefore, an understanding of Oct4 function in stem cell biology could also lead to novel treatments for certain malignancies.

References:

1. Pesce, M. & Scholer, H.: Mol. Rep. Dev. 55:452-7, 2000
2. Pesce, M. et al: BioEssays 20:722-32, 1998
3. Takahashi, K. & Yamanaka, S.: Cell 126:663-76, 2006
4. Tai, M-H. et al: Carcinogen. 26:495-502, 2006

TECHNICAL INFORMATION

Source:

Oct4 antibody is a rabbit antibody raised against a short peptide from human Oct4 sequence.

Specificity and Sensitivity:

This antibody detects endogenous Oct4 proteins without cross-reactivity with other family members.

Storage Buffer: Solution in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide.

Storage:

Store at -20°C for at least one year. Store at 4°C for frequent use. Avoid repeated freeze-thaw cycles.

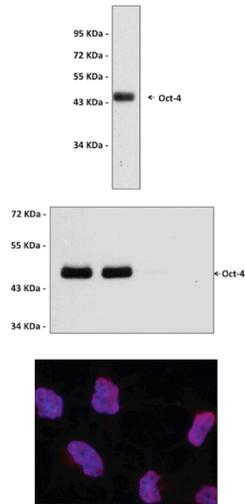
APPLICATIONS

Application:	*Dilution:
WB	1-2 ug/mL
IP	5-10 ug
IHC	n/d
ICC	n/d
FACS	n/d
ELISA	2-5 ug/mL

**Optimal dilutions must be determined by end user.*



QUALITY CONTROL DATA



Top: NT2/D1 whole cell extract was separated on SDS-PAGE and probed with 2 µg/mL Rabbit Anti-POU5F1 (Oct4). The antibody was developed using Goat Anti-Rabbit IgG-Peroxidase and a chemiluminescent substrate.

Middle: Rabbit Anti-POU5F1 (Oct-4) was used to immunoprecipitate POU5F1 (Oct-4) from NT2 cell lysate. Detection antibody: Anti-POU5F1(Oct-4). Lanes (left to right): 1.) 5 µg antibody. 2.) 10 µg antibody. 3.) Negative control – without cell lysate 4.) Negative control – without IP antibody

Bottom: Predominant Nuclear localization of POU5F1 (Oct-4) in F9 cells. Fixed cells were stained with 2 µg/mL Rabbit Anti-POU5F1 followed by Goat Anti-Rabbit, Cy3 conjugate, and counterstained with DAPI (blue) to stain nuclei (x100 magnification).

