



OCT4 antibody (other name: POU5F1, OTF4, OCT3, OTF3)

Cat. No.	C15410305	Specificity:	Human, mouse: positive.
Type:	Polyclonal		Other species: not tested. Affinity purified polyclonal antibody in PBS containing 0.05% azide and 0.05% ProClin
Source:	Rabbit	Purity:	
Lot:	A2089P		300.
Size:	50 µg	Storage:	Store at -20°C; for long storage, store at
Concentration:	1.5 µg/µl		-80°C. Avoid multiple freeze-thaw cycles.

Precautions: This product is for research use only. Not for use in diagnostic or therapeutic procedures.

Description: Polyclonal antibody raised in rabbit against OCT4 (Octamer-Binding Protein 4), using 4 KLH-conjugated synthetic peptides containing sequences from different parts of the protein.

Applications

Applications	Suggested dilution	References
ChIP*	5 µg/ChIP	Fig 1, 2
ELISA	1:1,000-10,000	Fig 3
Western blotting	1:1,000	Fig 4

Target description

OCT4 (UniProtKB/Swiss-Prot entry Q01860) is a transcription factor that plays a key role in embryonic development and stem cell pluripotency. During embryonic development OCT4 forms a trimeric complex with SOX2 and controls the expression of a number of genes important for embryogenesis such as YES1, FGF4, UTF1 and ZFP206. Aberrant expression of OCT4 in adult tissues is associated with tumorigenesis. A translocation with the Ewing's sarcoma gene on chromosome 21 also leads to tumor formation.

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Results



Figure 1. ChIP results obtained with the Diagenode antibody directed against OCT4

ChIP assays were performed using E14Tg2a mouse embryonic stem cells, the Diagenode antibody against OCT4 (Cat. No. C15410305) and optimized PCR primer sets for qPCR. ChIP was performed with the "iDeal ChIP-seq" kit (Cat. No. C01010055), using sheared chromatin from 4 million cells. A titration of the antibody consisting of 1, 2. 5 and 10 µg per ChIP experiment was analysed. IgG (2 µg/IP) was used as negative IP control. Quantitative PCR was performed with primers for known OCT4 targets UTF1, YES1 and ZSCAN10, used as positive control targets, and for the promoter of the GAPDH gene, used as a negative control. Figure 1 shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).



Figure 2. ChIP-seq results obtained with the Diagenode antibody directed against OCT4

ChIP was performed on sheared chromatin from 4 million E14Tg2a mouse embryonic stem cells using 5 µg of the Diagenode antibody against OCT4 (Cat. No. C15410305) as described above. The IP'd DNA was subsequently analysed on an Illumina HiSeq. Library preparation, cluster generation and sequencing were performed according to the manufacturer's instructions. The 51 bp tags were aligned to the mouse genome using the BWA algorithm. Figure 2 shows the peak distribution along the complete sequence of mouse chromosome 7 (figure 2A) and a 1Mb region containing the UTF1 positive control (figure 2B), and in a two genomic regions surrounding the YES1 and ZSCAN10 positive control genes (figure 2C and D).





Figure 3. Determination of the antibody titer

To determine the titer of the antibody, an ELISA was performed using a serial dilution of the Diagenode antibody directed against OCT4 (Cat. No. C15410305). The plates were coated with the peptides used for immunization of the rabbit. By plotting the absorbance against the antibody dilution (Figure 2), the titer of the antibody was estimated to be 1:100,000.



Figure 4. Western blot analysis using the Diagenode antibody directed against OCT4

Nuclear extracts (15 μ g) from human embryonic stem cells were analysed by Western blot using the Diagenode antibody against OCT4 (Cat. No. C15410305) diluted 1:1,000 in TBSTween containing 5% skimmed milk. The position of the protein of interest is indicated on the right; the marker (in kDa) is shown on the left.

