HOLOGIC[®] Diagenode

FOXA1

Cat. No. C15410231

Lot:	45308	Specificity:	Human: positive.
Size:	100 μΙ		Other species: not tested.
Туре:	Polyclonal ChIP/ChIP-seq grade	Purity:	Affinity purified polyclonal antibody
Isotype:	NA	Storage	PBS containing 20% glycerol, 1% BSA and 0.025% ProClin300.
Source:	Rabbit	buffer:	
Concentration:	0.44 μg/μl		

Storage: Store at -20°C; for long storage, store at -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 FOXA1 (Forkhead Box Protein A1), using a KLH coupled peptide from the C-terminal part of the protein.

Applications

Applications	Suggested dilution	References
ChIP*	2 μg per ChIP	Fig 1, 2
Western blotting	1:500 – 1:3,000	Fig 3
Immunoprecipitation	2.5 per IP	Fig 4
Immunofluorescence	1:100 – 1:1,000	Fig 5
Immunohistochemistry	1:100 – 1:1,000	Fig 6

*Please note that the optimal antibody amount per IP should be determined by the end-user. We recommend testing 1-5 µg per IP.

Target description

FOXA1 (UniProt/Swiss-Prot entry P55317) belongs to the forkhead class of transcription factors. FOXA1 was originally discovered as a transcriptional activator for liver-specific transcripts such as albumin and transthyretin. It is also involved in embryonic development, establishment of tissue-specific gene expression and regulation of gene expression in differentiated tissues, as well as in cell cycle regulation.

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Results



Figure 1: ChIP results obtained with the Hologic Diagenode antibody directed against FOXA1

ChIP assays were performed using HepG2 cells, the Hologic Diagenode antibody against FOXA1 (cat. No. C15410231) and optimized 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 and 5 μ g per ChIP experiment was analysed. IgG (1 μ g/IP) was used as negative IP control. QPCR was performed with primers for the FBXL18, RAD51B and TFF1 genes, used as positive controls, and for the Sat2 satellite repeat, 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 Hologic Diagenode antibody directed against FOXA1

ChIP was performed on sheared chromatin from 4 million HepG2 cells using 2 µg of the Hologic Diagenode antibody against FOXA1 (cat. No. C15410231) 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 50 bp tags were aligned to the human genome using the BWA algorithm. Figure 2 shows the enrichment along the complete sequence and an 800 kb region of human chromosome 1 (fig 2A and B), and in two genomic regions surrounding the FBXL18 and RAD51B positive control genes (fig 2C and D). The position of the amplicon used for qPCR ChIP validation is indicated by an arrow.





Figure 3: Western blot analysis using the Hologic Diagenode antibody directed against FOXA1

Nuclear extracts from HepG2 cells (30 μ g) were analysed by Western blot using the Hologic Diagenode antibody against FOXA1 (cat. No. C15410231) diluted 1:1,000. The position of the protein of interest is indicated on the right; the marker (in kDa) is shown on the left.



Figure 4: Immunoprecipitation using the Hologic Diagenode antibody directed against FOXA1

Immunoprecipitation was performed on whole cell extracts from HepG2 cells using 2.5 μ g of the Hologic Diagenode antibody against FOXA1 (cat. No. C15410231). An equal amount of rabbit IgG was used as a negative control. The immunoprecipitated FOXA1 protein was detected by western blot with the FOXA1 antibody diluted 1:1,000. The IP with the FOXA1 antibody and with the IgG negative control are shown in Iane 3 and Iane 2, respectively. Lane 1 shows the input (40 μ g of HepG2 whole cell extract).



Figure 5: Immunofluorescence with the Hologic Diagenode antibody directed against FOXA1

HeLa cells were fixed with formaldehyde and stained with the Hologic Diagenode antibody against FOXA1 (cat. C15410231) diluted 1:1,000 (left). The right picture shows costaining with Hoechst 33342 nucleic acid stain.





Figure 6: Immunohistochemistry using the Hologic Diagenode antibody directed against FOXA1

Formalin fixed paraffin embedded cervical carcinoma (fig 6A) or breast carcinoma (fig 6B) tissue was stained with the Hologic Diagenode antibody against FOXA1 (cat. No. C15410231) diluted 1:500 followed by a peroxidase labelled goat anti-rabbit secondary antibody.