TECHNICAL DATASHEET

LOT: 003

HOLOGIC® Diagenode

H3pan antibody

Cat. No. C15200011

Lot:	003
Size:	10 µg / 50 µg
Type:	Monoclonal, **ChIP-grade**
Isotype:	IgG3
Source:	Mouse
Concentration:	1.58 µg/µl

Specificity:	Human, mouse, maize, tomato, poplar, Arabidopsis, barley: positive. Other species: not tested.
Purity:	Protein A purified monoclonal antibody
Storage buffer:	PBS containing 0.05% azide.

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: Monoclonal antibody raised in mouse against histone H3, using a KLH-conjugated synthetic peptide containing an unmodified sequence from the C-terminus of the protein. This antibody can be used as a loading control in both ChIP and WB experiments.

Applications

Applications	Suggested dilution	References
ChIP*	1–2 µg per ChIP	Fig 1
Western blotting	1:1,000 – 1:5,000	Fig 2, 3
Immunofluorescence	1:500	Fig 4

*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

Histones are the main constituents of the protein part of chromosomes of eukaryotic cells. They are rich in the amino acids arginine and lysine and have been greatly conserved during evolution. Histones pack the DNA into tight masses of chromatin. Two core histones of each class (H2A, H2B, H3, and H4) assemble and are wrapped by 146 base pairs of DNA, forming one octameric nucleosome. Histones play a central role in the regulation of transcription, DNA repair, DNA replication and chromosomal stability. These different functions are established via a complex set of post-translational modifications which either directly or indirectly alter chromatin structure and DNA accessibility to facilitate transcriptional activation or repression or other nuclear processes.

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Results

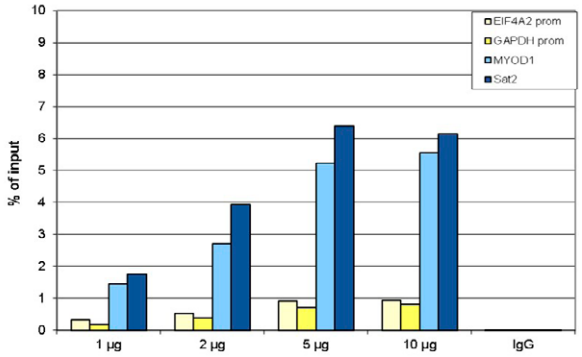

Figure 1: ChIP results obtained with the monoclonal antibody directed against H3

ChIP assays were performed using human K562 cells, the monoclonal antibody against H3 (cat. No. C15200011) and optimized PCR primer pairs for qPCR. ChIP was performed on sheared chromatin from 1 million cells. A titration consisting of 1, 2, 5 and 10 µg of antibody per ChIP experiment was analyzed. IgG (1 µg/IP) was used as a negative IP control. Quantitative PCR was performed with primers specific for the promoters of the active GAPDH and EIF4A2 genes, and for the inactive MYOD1 gene and the Sat2 satellite repeat. Figure 1 shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).

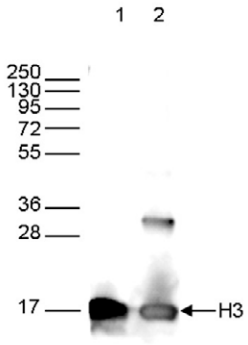

Figure 2: Western blot analysis using the monoclonal antibody directed against H3

Western blot was performed on whole cell extracts from HeLa cells (40 µg, lane 1) and on 1 µg of recombinant histone H3 (lane 2) using the monoclonal antibody against H3 (cat. No. C15200011). The antibody was diluted 1:2,000 in TBS-Tween containing 5% skimmed milk. The marker (in kDa) is shown on the left; the position of the protein of interest is indicated on the right.

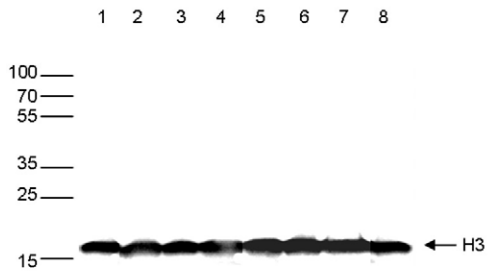

Figure 3: Western blot analysis using the monoclonal antibody directed against H3

Western blot was performed on whole cell extracts (30 µg) from different cell types (lane 1: HeLa, lane 2: K562, lane 3: MCF7, lane 4: U2OS, lane 5: HepG2, lane 6: Jurkat, lane 7: NIH3T3, lane 8: E14Tg2a mouse ES cells) using the monoclonal antibody against H3 (cat. No. C15200011). The antibody was diluted 1:1,000 in TBS-Tween containing 5% skimmed milk. The marker (in kDa) is shown on the left; the position of the protein of interest is indicated on the right.

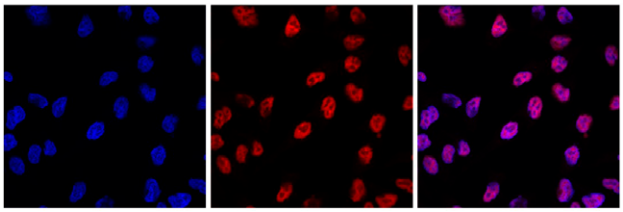

Figure 4: Immunofluorescence using the monoclonal antibody directed against H3

HeLa cells were stained with the monoclonal antibody against H3 (cat. No. C15200011) and with DAPI. Cells were fixed with 4% formaldehyde for 10' and blocked with PBS/TX-100 containing 1% BSA. The cells were immunofluorescently labelled with the H3 antibody (middle) diluted 1:500 in blocking solution followed by an anti-mouse antibody conjugated to Alexa594. The left panel shows staining of the nuclei with DAPI. A merge of the two stainings is shown on the right