N6-methyladenosine (m6A) polyclonal antibody

Cat. No. C15410208-50

Type: Polyclonal	Specificity: Human, mouse, other (wide range): positive.
Size: 50 µg	Isotype: NA
Concentration: 1.2 µg/µl	Host: Rabbit
Lot No.: A2125-0010	Purity: Protein G purified polyclonal antibody.
Storage buffer: PBS containing 0.05% azide and 0.05% ProClin 300.	Storage conditions: 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.	

Last Data Sheet Update: February 11, 2020

Description

Polyclonal antibody raised in rabbit against N6-methyladenosine (m6A) conjugated to LPH.

Applications

Applications	Suggested dilution	References
RIP*	1-2 µg per IP	Fig 1, 2, 3
Dot Blotting	1:400	Fig 4

*Please note that the optimal antibody amount per IP should be determined by the end-user. We recommend testing 1-10 µg per IP.

Target Description

N6-methyladenosine (m6A) is a modified base which is abundant in mRNA in most eukaryotes but also has been found in tRNA's, rRNA's, snRNA's and in long non-coding RNA's. Adenosine methylation is catalyzed by m6A methyltransferase, a large protein complex which has a preference for the consensus sequence GGACU. In human, the m6A modification has been identified in more than 7000 genes. It is preferably present around stop codons and in the 3' UTR but has not been observed in poly A tails. m6A is dynamically regulated both throughout development and in response to cellular stimuli. Levels are significantly higher in adulthood than during embryonic development. Although the presence of m6A in RNA was identified several years ago, it's physiological significance remains largely unknown. It has been proposed to affect mRNA processing and export from nucleus to cytoplasm. Recently, it was shown that mutations in the m6A demethylase gene FTO, which cause a decrease of m6A levels, are associated with an increased risk for obesity and type 2 diabetes.

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Validation data

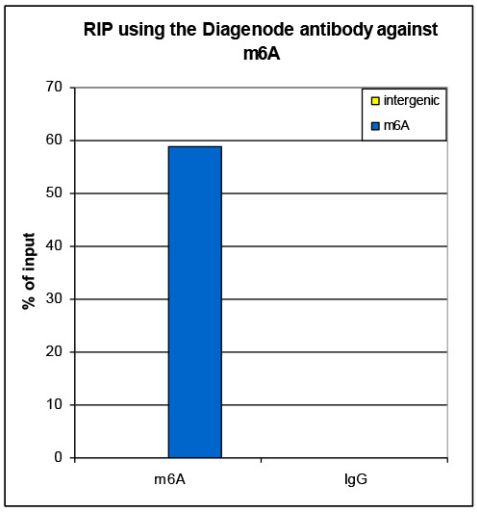


Figure 1. RNA immunoprecipitation using the Diagenode antibody directed against m6A

RNA Immunoprecipitation was performed on 40 µg HeLa total RNA spiked with 0.5 µg of an in vitro prepared transcript containing m6A nucleotides, using 2 µg of the Diagenode m6A antibody (Cat. No. C15410208). An equal amount of IgG was used as negative control. The immunoprecipitated RNA was subsequently analyzed by qRT-PCR with primers specific for the transcript and for an intergenic region, used as 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).

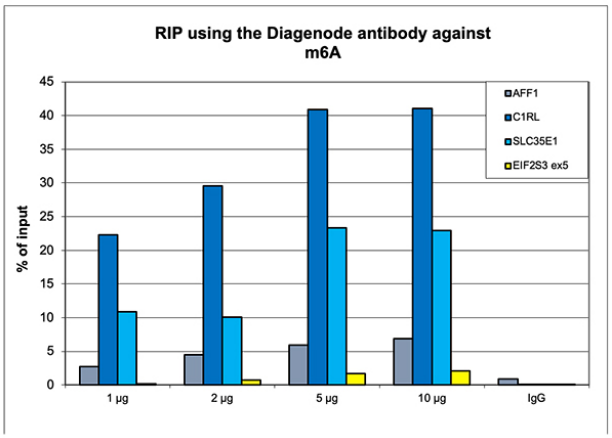
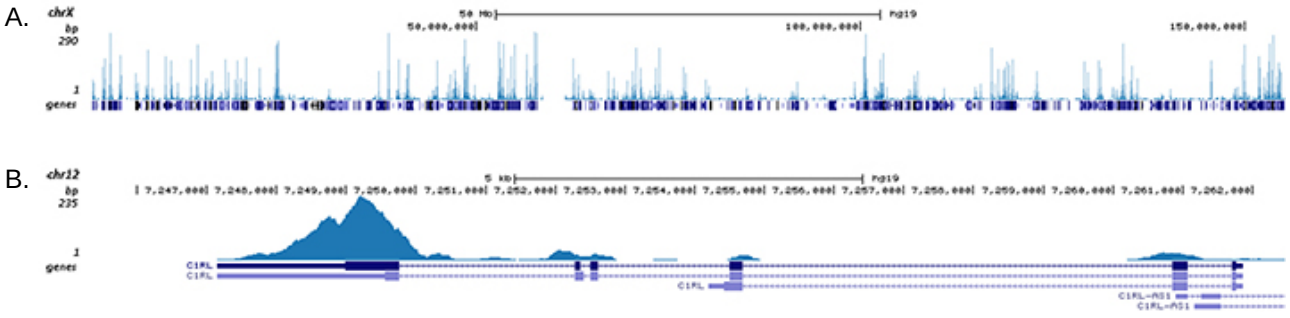


Figure 2. RNA immunoprecipitation using the Diagenode antibody directed against m6A

RNA Immunoprecipitation was performed on 40 µg HeLa total RNA fragmented to a mean size of ~500 bases. A titration consisting of 1, 2, 5 and 10 µg of antibody per RIP experiment was analyzed. IgG (2 µg/IP) was used as a negative IP control. Quantitative RT-PCR was performed with primers for 3' UTR of the C1RL and SLC35E1 genes, and for exon 12 of the AFF1 gene, used as positive controls, and for exon 5 of the EIF2S3 gene, used as 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).



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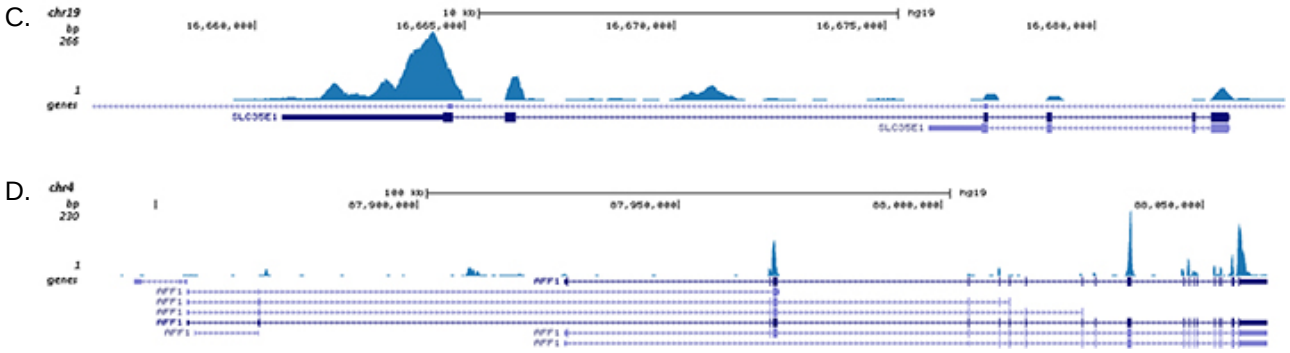

Figure 3. RIP-seq results obtained with the Diagenode antibody directed against m6A

RIP was performed with 2 µg of the Diagenode antibody against m6A (Cat. No. C15410208). The IP'd DNA was subsequently analysed on an Illumina HiSeq 4000. The 50 bp tags were aligned to the human genome using the BWA algorithm. Figure 3 shows the signal distribution along the complete sequence of the human X-chromosome (figure 3A) and in three genomic regions surrounding the C1RL, SLC35E1 and AFF1 positive control genes (figure 3B, C and D).

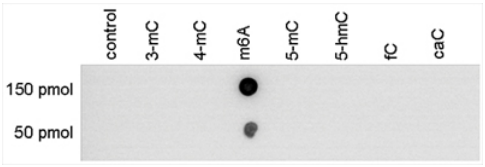

Figure 2. Dot blot analysis using the Diagenode antibody directed against m6A

To demonstrate the specificity of the Diagenode antibody against m6A (Cat. No. C15410208), a Dot Blot analysis was performed using synthetic oligonucleotides containing different modified bases. 150 and 50 pmol of the respective oligo's were spotted on the membrane. The antibody was diluted 1:400 in PBS-T containing 10 % skimmed milk and 1% BSA. Figure 1 shows a high specificity of the antibody for the oligonucleotide with the N6-methyladenosine modification.

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