diagenode
A Hologic Company

TECHNICAL DATASHEET

ac4C antibody

Cat. No. C15200252

Type: Monoclonal
Isotype: IgG1
Source: Mouse
Lot: 001
Size: 50 µg
Concentration: 0.7 µg/µl

Specificity: Human, other (wide range): positive.
Purity: Protein G purified monoclonal antibody.
Storage: Store at -20°C; for long storage, store at -80°C. Avoid multiple freeze-thaw cycles.
Storage buffer: PBS containing 0.05% sodium azide.

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

Description: Monoclonal antibody raised in mouse against 4-acetylcytosine (ac4C) conjugated to KLH.

Applications

Applications	Suggested dilution	References
RIP*	2 µg per IP	Fig 1
ELISA	1:1,000	Fig 2

*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

N4-acetylcytidine (ac4C) is the only acetylation that has been described in eukaryotic RNA. It's a highly conserved RNA modification which is present in mRNA, tRNA and rRNA. ac4C has been shown to have important regulatory functions and is involved in the regulation of mRNA stability and in RNA processing. Moreover, ac4C promotes translation efficiency.

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Results

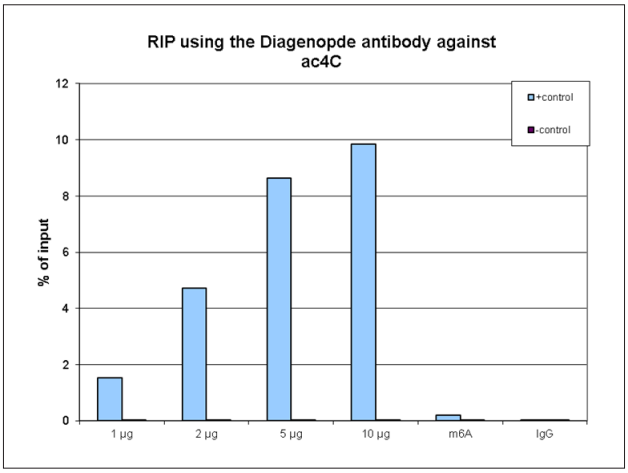

Figure 1. RNA immunoprecipitation using the Diagenode monoclonal antibody directed against ac4C

RNA immunoprecipitation (RIP) was performed on 40 µg total RNA isolated from HeLa cells using the Diagenode antibody against ac4C (cat. No. C15200252). The RNA was spiked with an in vitro produced RNA molecule containing ac4C nucleotides as well as an unmodified control RNA (300 ng each). A titration of the antibody consisting of 1, 2, 5 and 10 µg per RIP experiment was analysed. IgG as well as an antibody against m6A (C15410208) (both 2 µg/IP) were used as negative IP controls. QRT-PCR was performed with primers specific for the modified and unmodified RNA molecules. Figure 1 shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).

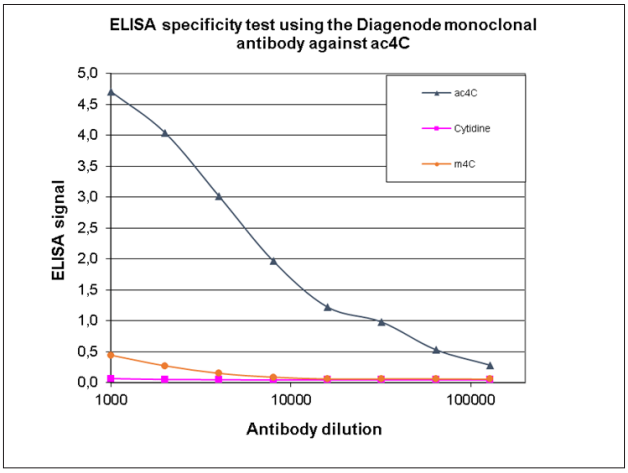

Figure 2. Cross reactivity of the Diagenode monoclonal antibody directed against ac4C

To test the specificity an ELISA was performed using a serial dilution of the Diagenode monoclonal antibody against ac4C (cat. No. C15200252). The wells were coated with the ac4C nucleoside as well as the m4C and unmodified Cytosine nucleosides coupled to BSA. Figure 2 shows a high specificity of the antibody for the modification of interest.