

## m66A Antibody

**Cat. No. C15410365**

Type: Polyclonal	Specificity: Human, mouse, other (wide range): positive
Size: 50 µg/21 µl	Isotype: NA
Concentration: 2.4 µg/µl	Host: Rabbit
Lot No.: A3001-0010	Purity: Protein A purified polyclonal antibody
Storage buffer: PBS containing 0.05% azide	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: May 7, 2020

### Description

Full name: **N6N6-dimethyladenosine polyclonal antibody**

Polyclonal antibody raised in rabbit against N6N6-dimethyladenosine (m66A) conjugated to BSA.

### Applications

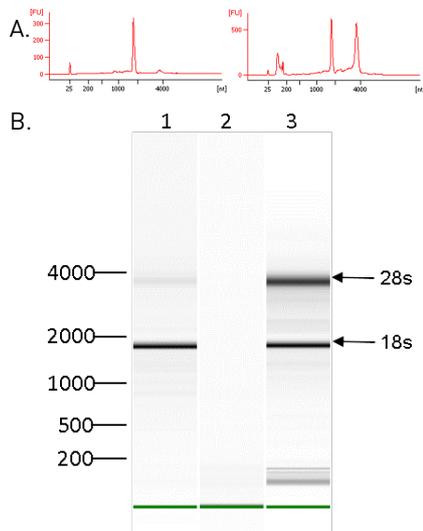
Applications	Suggested dilution	References
RIP *	1 - 2 µg per IP	Fig 1, 2
Dot Blotting	1:400	Fig 3

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

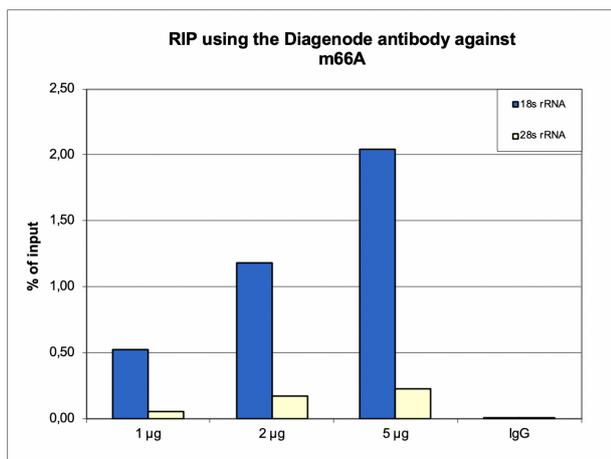
N6N6-dimethyladenosine (m66A) is a modified base which is mainly present in 18s rRNA.

**Validation data**



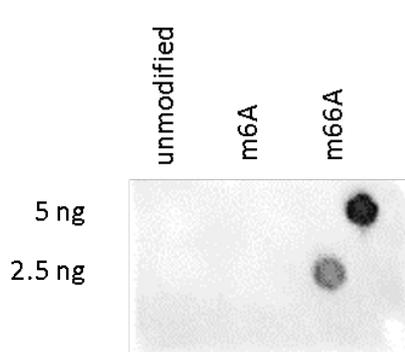
**Figure 1. RNA immunoprecipitation using the Diagenode antibody directed against m66A**

RNA immunoprecipitation (RIP) was performed on 40 µg total RNA isolated from HeLa cells using 2 µg of the Diagenode antibody against m66A (cat. No. C15410365) or with an equal amount of mouse IgG, used as a negative control. The immunoprecipitated RNA was subsequently analysed on a Bioanalyzer. Figure 1A shows the Bioanalyzer profile obtained with the m66A antibody (left) The right panel shows the input. Figure 1B shows the gel image for the m66A antibody, the IgG negative control and the input (lane 1, 2 and 3, respectively). The marker (in bp) is shown on the left, the position of the 28s and 18s ribosomal RNA is indicated on the right.



**Figure 2. RNA immunoprecipitation using the Diagenode antibody directed against m66A**

RIP assays were performed on 40 µg total RNA from human HeLa cells using the Diagenode m66A antibody (cat. nr. C15410365). A titration of the antibody consisting of 1, 2 and 5 µg per RIP experiment was analysed. IgG (2 µg/IP) was used as negative IP control. QRT-PCR was performed with primers for the 18s and 28s rRNA genes. Figure 2 shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).



**Figure 3. Dot blot analysis using the Diagenode antibody directed against m66A**

To demonstrate the specificity of the Diagenode antibody against m66A (cat. No. C15410365), a Dot Blot analysis was performed using in vitro transcribed RNA molecules containing m6A or m66A modified bases as well as an unmodified control RNA. 5 and 2.5 ng of the respective RNA molecules were spotted on the membrane. The antibody was diluted of 1:400 in TBS-T containing 10 % skimmed milk and 1% BSA. Figure 1 shows a high specificity of the antibody for the oligonucleotide with the N6N6-dimethyladenosine.