m6A (N6-methyladenosine) is a posttranscriptional RNA-modification found throughout all kingdoms, e.g. in vertebrate snRNAs U2, U4, U6, in viral and eukaryotic mRNAs, and in E. coli 16S rRNA. Recent studies have found that mRNA is predominately m6A modified at stop codons and long internal exons, which are conserved between mouse and human. The so-called RNA methylome probably plays an important role in the regulation of gene expression. In E. coli, Dam methylase introduces m6A modifications on the DNA level at the 5’-GATC-3’ motif. This allows the cell to differentiate between the parental and the daughter strand during mismatch repair.

## Selected References SYSY Antibodies

**N6-methyladenine DNA Modification in Glioblastoma.**

**MadID, a Versatile Approach to Map Protein-DNA Interactions, Highlights Telomere-Nuclear Envelope Contact Sites in Human Cells.**
Sobeki M, Souaid C, Boulaj Y, Guerineau V, Noordermeer D, Crabbe L

**Immuno-Northern Blotting: Detection of RNA Modifications by Using Antibodies against Modified Nucleosides.**

**Transient Focal Ischemia Significantly Alters the m6A Epitranscriptomic Tagging of RNAs in the Brain.**
Stroke (2019) : STROKEAHA19026433. DOTBLOT, IP; tested species: mouse

**Identification of a DNA N6-Adenine Methyltransferase Complex and Its Impact on Chromatin Organization.**
Beh LY, Dehou tranca GT, Clay DM, Thompson RE, Lindblad KA, Hutten ER, Brach JS, Sebra RP, Muir TW, Landweber LF
Cell (2019) : DOTBLOT, IP

**DOTBLOT, IHC-P; tested species: human**
Mett13-mediated mRNA m6A methylate promotes dendritic cell activation.

**Small-Molecule Targeting of Oncogenic FTO Dimethylase in Acute Myeloid Leukemia.**

**Evolution of the RNA N6-methyladenosine methylome mediated by genomic duplication.**
Miao Z, Zhang T, Qi Y, Song J, Han Z, Ma C
Plant physiology (2019) : DOTBLOT, IP

**DOTBLOT, IHC-P; tested species: human**
METTL3 and ALKBH5 oppositely regulate m6A modification of TFEB mRNA, which dictates the fate of hypoxia/reoxygenation-treated cardiomyocytes.

hIV-1 envelope proteins up-regulate N6-methyladenosine levels of cellular RNA independently of viral replication.
Tirumuru N, Wu L

**N6-Methyladenosine Guided mRNA Alternative Translation during Integrated Stress Response.**

**DNA N6-Adenine Methylation in Arabidopsis thaliana.**

**Recognition of RNA N6-methyladenosine by IGF2BP proteins enhances mRNA stability and translation.**

**R-2HG Exhibits Anti-tumor Activity by Targeting FTO/m6A/MYC/CEBP Signaling.**

**N6-methyladenosine RNA modification regulates embryonic neural stem cell self-renewal through histone modifications.**

**A dynamic N6-methyladenosine methylome regulates intrinsic and acquired resistance to tyrosine kinase inhibitors.**
Cell research (2018) : DOTBLOT, IP; tested species: human

### Data Sheet

**m6A (N6-methyladenosine)** is a posttranscriptional RNA-modification found throughout all kingdoms, e.g. in vertebrate snRNAs U2, U4, U6, in viral and eukaryotic mRNAs, and in E. coli 16S rRNA. Recent studies have found that mRNA is predominately m6A modified at stop codons and long internal exons, which are conserved between mouse and human. The so-called RNA methylome probably plays an important role in the regulation of gene expression. In E. coli, Dam methylase introduces m6A modifications on the DNA level at the 5’-GATC-3’ motif. This allows the cell to differentiate between the parental and the daughter strand during mismatch repair.

#### Reconstitution/Storage
50 µg specific antibody, lyophilized. Affinity purified with the immunogen. Albumin was added for stabilization. For reconstitution add 50 µl H2O to get a 1mg/ml solution in PBS. Then aliquot and store at -20°C until use.

#### Applications
- **WB**: 1 : 1000 up to 1 : 10000 (AP staining) suitable for Dot Blot
- **IP**: yes (see remarks)
- **ICC**: 1 : 200
- **IHC**: not tested yet
- **IHC-P/FPFE**: 1 : 100
- **ELISA**: yes suitable for sandwich-ELISA

#### Immunogen
N6-methyladenosine fused to BSA.

#### Reactivity
Reacts with: human, rat, mouse, eukaryotes, prokaryotes. Other species not tested yet.

#### Specificity
Specific for N6-methyladenosine (m6A) with some cross-reactivity to m6Am.

#### Remarks
- **IP**: Extracts from eukaryotic and prokaryotic cells. Standard protocol for the IP of nuclear extracts is provided with the product. For the isolation of m6A modified RNA from total or mRNA preparations the protocol according to Dominissini et al. (2013) is recommended.
- **ELISA**: yes suitable for sandwich-ELISA

**TO BE USED IN VITRO / FOR RESEARCH ONLY**

**NOT TOXIC, NOT HAZARDOUS, NOT INFECTIOUS, NOT CONTAGIOUS**
Anti- m6A (N6-methyladenosine)

rabbit polyclonal affinity purified antibody; cat. no. 202 003

Standard Protocol for Immunoprecipitation

1. 10 - 15 µg antibody per assay are coupled to protein A or protein G-sepharose in PBS-buffer at 4 °C head over tail (several hours).
2. The pellet is washed three times with ice-cold PBS.
3. Incubate immobilized antibody with extract in appropriate buffer for 1 hour on a head over tail rotor at 4°C. The buffer should be optimized to your needs, i.e. the investigated complexes should be stable in the buffer. The buffer should provide stringency to avoid non-specific interaction, e.g. 20 µl of HeLa nuclear extract in 250 µl IPP buffer (Tris-HCL, pH 7.4, 150 mM NaCl, 0.1 % NP40). Generally, non-specific interactions should be controlled with a parallel pull-down assay using protein A/G-sepharose without antibody.
4. Wash five times with one ml of buffer. Usually, the buffer used for washing is identical with the incubation buffer in step 3. After two washes the content of the reaction tube should be transferred to a new one. This step significantly reduces background in pull-down assays.
5. The pellet-bound RNA can be isolated by shaking the tube with 250 µl of buffer with one volume of phenol/chloroform and subsequent ethanol precipitation of the aqueous phase. Alternatively, the precipitated RNA-(complex) may be eluted by shaking with 250 µl of RNA-elution buffer (e.g. Tris-HCL, pH 7.4, 450 mM NaCl, 0.4% SDS). After phenol/chloroform-extraction of the eluate protein and RNA-containing phases are precipitated and subjected to analysis.
6. RNA-analysis: native RNA may be analyzed by 3’-terminal pCp-labelling or Northern.
7. pCp-Labelling: take care that the RNA-pellet is free of residual phenol by washing the pellet twice with 80% ethanol. Dry pellet in a sped-vac and incubate with 10 µl of reaction mixture at 4 °C over night (e.g. in a fridge).

Reaction mixture (10 µl/assay):

1 µl 10X T4 RNA Ligase buffer (e.g. New England Biolabs)
0.5 µl T4 RNA Ligase (e.g. New England Biolabs)
2.5 µl DMSO
1 µl RNAse- Inhibitor (recommended)
5 µl pCp (Amersham/Pharmacia)

The reaction mixtures may be loaded directly on a denaturing polyacrylamid gel. It should be noted, however, that occasionally upon direct loading additional bands can appear. To avoid such gel artifacts, a phenol/chloroform extraction may be performed.