TO BE USED IN VITRO / FOR RESEARCH ONLY
NOT TOXIC, NOT HAZARDOUS, NOT INFECTIOUS, NOT CONTAGIOUS

m6A (N6-methyladenosine) is a posttranscriptional RNA-modification found throughout all kingdoms, e.g. in vertebrate snmRNAs 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 methylole 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 probably plays an important role in the regulation of gene expression. Internal exons, which are conserved between mouse and human. The so-called RNA methylome allows the cell to differentiate between the parental and the daughter strand during mismatch repair. Synaptic N6-methyladenosine (m6A) epitranscriptome reveals functional partitioning of localized transcripts. Neurons.

Selected References SYSY Antibodies


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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):

<table>
<thead>
<tr>
<th>Volume</th>
<th>Component</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 µl</td>
<td>10X T4 RNA Ligase buffer (e.g. New England Biolabs)</td>
<td></td>
</tr>
<tr>
<td>0.5 µl</td>
<td>T4 RNA Ligase (e.g. New England Biolabs)</td>
<td></td>
</tr>
<tr>
<td>2.5 µl</td>
<td>DMSO</td>
<td></td>
</tr>
<tr>
<td>1 µl</td>
<td>RNAse- Inhibitor (recommended)</td>
<td></td>
</tr>
<tr>
<td>5 µl</td>
<td>pCp (Amersham/Pharmacia)</td>
<td></td>
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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.