Cat.No. 201 001; Monoclonal mouse antibody, 100 µl ascites (lyophilized)

**Data Sheet**

| Reconstitution/Storage | 100 µl ascites, lyophilized. For reconstitution add 100 µl H2O, then aliquot and store at -20°C until use. |
| Applications           | WB: not recommended IP: yes (see remarks) ICC: yes (see reference) IHC: not tested yet IHC-P/FFPE: not tested yet |
| Clone                  | H20 |
| Subtype                | IgG1 (κ light chain) |
| Immunogen             | Synthetic m3G-cap conjugated to human serum albumin. |
| Reactivity             | Reacts with: human, rat, mouse, eukaryotes. Other species not tested yet. |
| Specificity            | Recognizes m3G-cap and m7G-cap. |
| Remarks                | This antibody can be used to detect capped RNAs (e.g. in viruses) or to identify and purify proteins associated with capped RNAs (see reference #2). IP: Human extracts or extracts from Xenopus laevis. Standard protocol provided with the product. |

**Selected References SYSY Antibodies**

mRNA Capping by Venezuelan Equine Encephalitis Virus nsP1: Functional Characterization and Implications for Antiviral Research.

RNA-methylation-dependant RNA processing controls the speed of the circadian clock.

MAPCap allows high-resolution detection and differential expression analysis of transcription start sites.

Microprocessor mediates transcriptional termination of long noncoding RNA transcripts hosting microRNAs.

XRN1 and the 5’ UTR of Hepatitis C virus and Bovine Viral Diarrhea virus is associated with dysregulated host mRNA stability.

Noncoding RNAs and LRF1P1 regulate TNF expression.

Stress-induced IncRNAs evade nuclear degradation and enter the translational machinery.

The eIF4E-binding protein Eap1p functions in Vts1p-mediated transcript decay.

Identification of a cytoplasmic complex that adds a cap onto 5’-monophosphate RNA.

Induced ncRNAs allosterically modify RNA-binding proteins in cis to inhibit transcription.

The interaction between cap-binding complex and RNA export factor is required for intronless mRNA export.

The imprinted Air ncRNA is an atypical RNAPII transcript that evades splicing and escapes nuclear export.
Seidl CI, Stricker SH, Barlow DP | The EMBO journal (2006) 25(15): 3565-75. IP |

Snurportin1, an m3G-cap-specific nuclear import receptor with a novel domain structure.

A monoclonal antibody against 2,2',7-trimethylguanosine that reacts with intact, class U, small nuclear ribonucleoproteins as well as with 7-methylguanosine-capped RNAs.

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Selected General References

A monoclonal antibody against 2,2',7-trimethylguanosine that reacts with intact, class U, small nuclear ribonucleoproteins as well as with 7-methylguanosine-capped RNAs.

Identification of Methylated Deoxycadenosines in Genomic DNA by dA6m DNA Immunoprecipitation.

mRNA Capping by Venezuelan Equine Encephalitis Virus nsP1: Functional Characterization and Implications for Antiviral Research.
Anti- m₃G- / m⁷G-cap

mouse monoclonal antibody (Cl. H 20); ascites; cat. no. 201 001

Standard Protocol for Immunoprecipitation

1. 10 µl of ascites per assay are coupled to 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 tubes 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.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.