Synaptic Systems

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m3G-cap, m7G-cap

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

Data Sheet

Reconstitution/ Storage	100 µl ascites, lyophilized. For reconstitution add 100 µl H_2O, 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 m_3G-cap conjugated to human serum albumin.
Reactivity	Reacts with: human, rat, mouse, eukaryotes. Other species not tested yet.
Specificity	Recognizes m_3G-cap and m^7G-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.

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

Polymerase II transcripts contain a 5´-terminal **m^7G-cap** that is required for the export of these transcripts from the nucleus to the cytoplasm and eucaryotic translation initiation. The Polymerase II transcribed spliceosomal snRNAs U1, U2, U4 and U5 assemble with the eight Sm proteins B/B´, D1, D2, D3, E, F, and G thus forming a core-UsnRNP. The core-UsnRNP is recognized by a methyltransferase that introduces two additional methyl groups to the m^7G-cap thus forming the **m_3G-cap** (hypermethylation). The m_3G-cap forms one part of the bipartite nuclear localisation signal (NLS) of the UsnRNPs. It is thus necessary for the nuclear re-import of the core-UsnRNPs. Also certain snoRNAs that are involved in the processing of pre-rRNAs contain an m_3G-cap.

Selected References SYSY Antibodies

mRNA Capping by Venezuelan Equine Encephalitis Virus nsP1: Functional Characterization and Implications for Antiviral Research.
Li C, Guillén J, Rabah N, Blanjoie A, Debart F, Vasseur JJ, Canard B, Decroly E, Coutard B
Journal of virology (2015) 89(16): 8292-303. **WB**

RNA-methylation-dependent RNA processing controls the speed of the circadian clock.
Fustin JM, Doi M, Yamaguchi Y, Hida H, Nishimura S, Yoshida M, Isagawa T, Morioka MS, Kakeya H, Manabe I, Okamura H, et al.
Cell (2013) 155(4): 793-806. **IP**

Microprocessor mediates transcriptional termination of long noncoding RNA transcripts hosting microRNAs.
Dhir A, Dhir S, Proudfoot NJ, Jopling CL
Nature structural & molecular biology (2015) 22(4): 319-27. **IP**

XRN1 stalling in the 5' UTR of Hepatitis C virus and Bovine Viral Diarrhea virus is associated with dysregulated host mRNA stability.
Moon SL, Blackinton JG, Anderson JR, Dozier MK, Dodd BJ, Keene JD, Wilusz CJ, Bradrick SS, Wilusz J
PLoS pathogens (2015) 11(3): e1004708. **IP**

Noncoding RNAs and LRRFIP1 regulate TNF expression.
Shi L, Song L, Fitzgerald M, Maurer K, Bagashev A, Sullivan KE
Journal of immunology (Baltimore, Md. : 1950) (2014) 192(7): 3057-67. **IP; tested species: human**

Stress-induced lncRNAs evade nuclear degradation and enter the translational machinery.
Galipon J, Miki A, Oda A, Inada T, Ohta K
Genes to cells : devoted to molecular & cellular mechanisms (2013) 18(5): 353-68. **IP; tested species: fission yeast**

The eIF4E-binding protein Eap1p functions in Vts1p-mediated transcript decay.
Rendl LM, Bieman MA, Vari HK, Smibert CA
PloS one (2012) 7(10): e47121. **IP**

Identification of a cytoplasmic complex that adds a cap onto 5'-monophosphate RNA.
Otsuka Y, Kedersha NL, Schoenberg DR
Molecular and cellular biology (2009) 29(8): 2155-67. **IP**

Induced ncRNAs allosterically modify RNA-binding proteins in cis to inhibit transcription.
Wang X, Arai S, Song X, Reichart D, Du K, Pascual G, Tempst P, Rosenfeld MG, Glass CK, Kurokawa R
Nature (2008) 454(7200): 126-30. **IP**

The interaction between cap-binding complex and RNA export factor is required for intronless mRNA export.
Nojima T, Hirose T, Kimura H, Hagiwara M
The Journal of biological chemistry (2007) 282(21): 15645-51. **IP**

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.
Huber J, Cronshagen U, Kadokura M, Marshallsay C, Wada T, Sekine M, Lührmann R
The EMBO journal (1998) 17(14): 4114-26. **IP**

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.
Bochnig P, Reuter R, Bringmann P, Lührmann R
European journal of biochemistry (1987) 168(2): 461-7. **IP**

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.
Bochnig P, Reuter R, Bringmann P, Lührmann R
European journal of biochemistry (1987) 168(2): 461-7.

Identification of Methylated Deoxyadenosines in Genomic DNA by dA6m DNA Immunoprecipitation.
Koziol MJ, Bradshaw CR, Allen GE, Costa AS, Frezza C
Bio-protocol (2016) 6(21): .

mRNA Capping by Venezuelan Equine Encephalitis Virus nsP1: Functional Characterization and Implications for Antiviral Research.
Li C, Guillén J, Rabah N, Blanjoie A, Debart F, Vasseur JJ, Canard B, Decroly E, Coutard B
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Anti- m_3G- / m^7G-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.