SYSY Antibodies

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m3G-cap, m7G-cap

Cat.No. 201 011; Monoclonal mouse antibody, 100 µg purified IgG (lyophilized)

Data Sheet

Reconstitution/ Storage	100 µg purified IgG, lyophilized. Albumin and azide were added for stabilization. For **reconstitution** add 100 µl H_2O to get a 1mg/ml solution in PBS. Then aliquot and store at -20°C to -80°C until use. Antibodies should be stored at +4°C when still lyophilized. Do not freeze! For detailed information, see back of the data sheet.
Applications	**WB**: not recommended **IP**: yes **IHC**: not tested yet **IHC-P**: 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).

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

Background

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 for 201 011

Approved drugs screening against the nsP1 capping enzyme of Venezuelan equine encephalitis virus using an immuno-based assay.
Ferreira-Ramos AS, Li C, Eydoux C, Contreras JM, Morice C, Quérat G, Gigante A, Pérez Pérez MJ, Jung ML, Canard B, Guillemot JC, et al.
Antiviral research (2019) : . . **WB, ELISA**

The NSL complex-mediated nucleosome landscape is required to maintain transcription fidelity and suppression of transcription noise.
Lam KC, Chung HR, Semplicio G, Iyer SS, Gaub A, Bhardwaj V, Holz H, Georgiev P, Akhtar A
Genes & development (2019) 337-8: 452-465. . **IP; tested species: drosophila**

PABP/purine-rich motif as an initiation module for cap-independent translation in pattern-triggered immunity.
Wang J, Zhang X, Greene GH, Xu G, Dong X
Cell (2022) : . . **IP**

CD47 interactions with exportin-1 limit the targeting of m7G-modified RNAs to extracellular vesicles.
Kaur S, Saldana AC, Elkahloun AG, Petersen JD, Arakelyan A, Singh SP, Jenkins LM, Kuo B, Reginauld B, Jordan DG, Tran AD, et al.
Journal of cell communication and signaling (2021) : . . **IP; tested species: human**

Capping pores of alphavirus nsP1 gate membranous viral replication factories.
Jones R, Bragagnolo G, Arranz R, Reguera J
Nature (2020) : . . **WB**

Development of RNA aptamer that inhibits methyltransferase activity of dengue virus.
Jung JI, Han SR, Lee SW
Biotechnology letters (2018) 402: 315-324. . **WB**

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 et al. Eur. J. Biochem. (1987) PubMed:2959477

Identification of Methylated Deoxyadenosines in Genomic DNA by dA6m DNA Immunoprecipitation.
Koziol MJ et al. Bio Protoc (2016) PubMed:28180135

mRNA Capping by Venezuelan Equine Encephalitis Virus nsP1: Functional Characterization and Implications for Antiviral Research.
Li C et al. J. Virol. (2015) PubMed:26041283

XRN1 stalling in the 5' UTR of Hepatitis C virus and Bovine Viral Diarrhea virus is associated with dysregulated host mRNA stability.
Moon SL et al. PLoS Pathog. (2015) PubMed:25747802

Access the online factsheet including applicable protocols at https://sysy.com/product/201011 or scan the QR-code.

FAQ - How should I store my antibody?

Shipping Conditions

- All our antibodies and control proteins / peptides are shipped lyophilized (vacuum freeze-dried) and are stable in this form without loss of quality at ambient temperatures for several weeks.

Storage of Sealed Vials after Delivery

- **Unlabeled** and **biotin-labeled antibodies** and **control proteins** should be stored at 4°C before reconstitution. **They must not be stored in the freezer when still lyophilized!** Temperatures below zero may cause loss of performance.
- **Fluorescence-labeled antibodies** should be reconstituted immediately upon receipt. Long term storage (several months) may lead to aggregation.
- **Control peptides** should be kept at -20°C before reconstitution.

Long Term Storage after Reconstitution (General Considerations)

- The storage freezer must not be of the frost-free variety ("no-frost freezer"). This cycle between freezing and thawing (to reduce frost-build-up), which is exactly what should be avoided. For the same reason, antibody vials should be placed in an area of the freezer that has minimal temperature fluctuations, for instance towards the back rather than on a door shelf.
- Aliquot the antibody and store frozen (-20°C to -80°C). Avoid very small aliquots (below 20 µl) and use the smallest storage vial or tube possible. The smaller the aliquot, the more the stock concentration is affected by evaporation and adsorption of the antibody to the surface of the storage vial or tube. Adsorption of the antibody to the surface leads to a substantial loss of activity.
- The addition of glycerol to a final concentration of 50% lowers the freezing point of your stock and keeps your antibody at -20°C in liquid state. This efficiently avoids freeze and thaw cycles.

Product Specific Hints for Storage

Control proteins / peptides

- Store at -20°C to -80°C.

Monoclonal Antibodies

- **Ascites** and **hybridoma supernatant** should be stored at -20°C up to -80°C. **Prolonged storage at 4°C is not recommended!** Unlike serum, ascites may contain proteases that will degrade the antibodies.
- **Purified IgG** should be stored at -20°C up to -80°C. Adding a carrier protein like BSA will increase long term stability. Many of our antibodies already contain carrier proteins. Please refer to the data-sheet for detailed information.

Polyclonal Antibodies

- **Crude antisera**: With anti-microbials added, they may be stored at 4°C. However, frozen storage (-20°C up to -80°C) is preferable.
- **Affinity purified antibodies**: Less robust than antisera. Storage at -20°C up to -80°C is recommended. Adding a carrier protein like BSA will increase long term stability. Most of our antibodies already contain carrier proteins. Please refer to the data-sheet for detailed information.

Fluorescence-labeled Antibodies

- Store as a liquid with 1 : 1 (v/v) glycerol at -20°C. Protect these antibodies from light exposure.

Avoid repeated freeze-thaw cycles for all antibodies!

FAQ - How should I reconstitute my antibody?

Reconstitution

- All our purified antibodies are lyophilized from PBS. To reconstitute the antibody in PBS, add the amount of deionized water given in the respective datasheet. If higher volumes are preferred, add water as mentioned above and then the desired amount of PBS and a stabilizing carrier protein (e.g. BSA) to a final concentration of 2%. Some of our antibodies already contain albumin. Take this into account when adding more carrier protein.
  For complete reconstitution, carefully remove the lid. After adding water, briefly vortex the solution. You can spin down the liquid by placing the vial into a 50 ml centrifugation tube filled with paper.
- If desired, add small amounts of azide or thimerosal to prevent microbial growth. This is especially recommended if you want to keep an aliquot a 4°C.
- After reconstitution of fluorescence-labeled antibodies, add 1 : 1 (v/v) glycerol to a final concentration of 50%. This lowers the freezing point of your stock and keeps your antibody in liquid state at -20°C.
- Glycerol may also be added to unlabeled primary antibodies. It is a suitable way to avoid freeze-thaw cycles.
- Please refer to our **tips and hints for subsequent storage** of reconstituted antibodies and control peptides and proteins.