SYSY Antibodies

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m6A

Cat.No. 202 018; Recombinant rabbit antibody, 100 µg recombinant IgG (lyophilized)

Data Sheet

Reconstitution/ Storage	100 µg purified recombinant 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	**Dot blot**: 1 : 1000 up to 1 : 2000 **IP**: yes **MeRIP**: yes
Clone	Rb345E11
Subtype	IgG1 (κ light chain)
Immunogen	N6-methyladenosine fused to BSA.
Reactivity	Reacts with: human, rat, mouse, prokaryotes, eukaryotes. Other species not tested yet.
Specificity	Specific for N6-methyladenosine (m6A) with some cross-reactivity to m6Am
Remarks	This antibody is a chimeric antibody based on the well known monoclonal mouse antibody clone 345E11. The constant regions of the heavy and light chains have been replaced by rabbit specific sequences. Therefore, the antibody can be used with standard anti-rabbit secondary reagents. The antibody has been expressed in mammalian cells.

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

Background

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 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 General References

Antibodies specific for N6-methyladenosine react with intact snRNPs U2 and U4/U6.
Bringmann P et al. FEBS Lett. (1987) PubMed:2951275

RNA m6A methylation regulates the ultraviolet-induced DNA damage response.
Xiang Y et al. Nature (2017) PubMed:28297716

Human METTL16 is a N6-methyladenosine (m6A) methyltransferase that targets pre-mRNAs and various non-coding RNAs.
Warda AS et al. EMBO Rep. (2017) PubMed:29051200

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

Identification of methylated deoxyadenosines in vertebrates reveals diversity in DNA modifications.
Koziol MJ et al. Nat. Struct. Mol. Biol. (2016) PubMed:26689968

N6-Methyladenosine in Flaviviridae Viral RNA Genomes Regulates Infection.
Gokhale NS et al. Cell Host Microbe (2016) PubMed:27773535

Mouse Maternal High-Fat Intake Dynamically Programmed mRNA m⁶A Modifications in Adipose and Skeletal Muscle Tissues in Offspring.
Li X et al. Int J Mol Sci (2016) PubMed:27548155

m(6)A-LAIC-seq reveals the census and complexity of the m(6)A epitranscriptome.
Molinie B et al. Nat. Methods (2016) PubMed:27376769

Widespread occurrence of N6-methyladenosine in bacterial mRNA.
Deng X et al. Nucleic Acids Res. (2015) PubMed:26068471

m(6)A RNA methylation is regulated by microRNAs and promotes reprogramming to pluripotency.
Chen T et al. Cell Stem Cell (2015) PubMed:25683224

N6-methyladenosine marks primary microRNAs for processing.
Alarcón CR et al. Nature (2015) PubMed:25799998

N6-adenosine methylation in MiRNAs.
Berulava T et al. PLoS ONE (2015) PubMed:25723394

DNA Methylation on N6-Adenine in C. elegans.
Greer EL et al. Cell (2015) PubMed:25936839

Decomposition of RNA methylome reveals co-methylation patterns induced by latent enzymatic regulators of the epitranscriptome.
Liu L et al. Mol Biosyst (2015) PubMed:25370990

Stem cells. m6A mRNA methylation facilitates resolution of naïve pluripotency toward differentiation.
Geula S et al. Science (2015) PubMed:25569111

N(6)-methyladenosine-dependent RNA structural switches regulate RNA-protein interactions.
Liu N et al. Nature (2015) PubMed:25719671

Access the online factsheet including applicable protocols at https://sysy.com/product/202018 or scan the QR-code.

FAQ - How should I store my antibody?

Shipping Conditions

- All SYSY antibodies and control proteins/peptides are shipped lyophilized (vacuum freeze-dried). In this form, they remain stable 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. **Do not freeze lyophilized antibodies**. Temperatures below 0°C may impair performance.
- **Fluorescence-labeled antibodies** should be reconstituted immediately upon receipt. Long-term storage of lyophilized fluorophore-conjugates may cause aggregation.
- **Control peptides** should be stored at -20°C before reconstitution.

Long Term Storage after Reconstitution (General Considerations)

- **Do not use frost-free ("no-frost") freezers.** These units periodically warm to remove ice buildup, causing freeze–thaw cycles that can damage antibodies.
- Store vials in areas with minimal temperature fluctuation - preferably toward the back of the freezer, not on the door.
- Aliquot reconstituted antibodies and store at –20°C to –80°C.
- Avoid very small aliquots (<20 µL), as evaporation and adsorption to tube surfaces can reduce antibody concentration and activity.
- Use the smallest practical storage vial to minimize surface area.
- Adding glycerol to a final concentration of 50% prevents freezing at -20°C, allowing storage in liquid form and effectively avoiding freeze–thaw cycles.

Product Specific Hints for Storage

Control proteins / peptides

- Store at -20°C to -80°C

Monoclonal Antibodies

- **Ascites and hybridoma supernatant:** Store at -20°C to -80°C
  Prolonged storage at 4°C is not recommended, as proteases present in ascites may degrade antibodies.
- **Purified IgG:** Store at -20°C to -80°C
  Adding a carrier protein (e.g., BSA) enhances long-term stability.
  Many SYSY antibodies already contain carrier proteins - refer to the respective datasheet for details.

Polyclonal Antibodies

- **Crude antisera:** Can be stored at 4°C with antimicrobials added, but -20°C to -80°C is preferred
- **Affinity-purified antibodies:** Less stable than antisera; store at -20°C to -80°C
  Adding a carrier protein such as BSA improves long-term stability.
  Most SYSY antibodies already contain carrier proteins - refer to the respective datasheet for details.

Fluorescence-labeled Antibodies

- Store as a liquid with 1:1 (v/v) glycerol at -20°C, and protect from light exposure

Avoid repeated freeze-thaw cycles for all antibodies!

FAQ - How should I reconstitute my antibody?

Reconstitution

- All purified SYSY antibodies are lyophilized from PBS. To reconstitute the antibody in PBS, add the volume of deionized water specified in the corresponding datasheet. If a larger final volume is desired, first add the recommended amount of water, then adjust with PBS and, if needed, add a stabilizing carrier protein (e.g., BSA) to a final concentration of 2%. Some SYSY antibodies already contain albumin; please take this into account before adding additional carrier protein.

For complete reconstitution, carefully remove the vial cap. After adding water, briefly vortex the solution. To collect the liquid at the bottom of the vial, place the vial inside a 50 ml centrifuge tube padded with paper and centrifuge briefly.

- If desired, small amounts of azide or thimerosal may be added to prevent microbial growth. This is particularly recommended when storing an aliquot at 4°C.
- After reconstitution of fluorescence-labeled antibodies, add glycerol 1:1 (v/v) to achieve a final concentration of 50%. This prevents freezing at –20°C and keeps the antibody in liquid form, effectively avoiding freeze–thaw cycles.
- Glycerol may also be added to unlabeled primary antibodies as a general measure to prevent freeze–thaw damage.
- For further guidance, please refer to our **storage tips** and recommendations for reconstituted antibodies, control peptides, and control proteins.