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 The constant regions of the heavy and light chains have been replaced by rabbit specific sequences.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 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.