

m6A

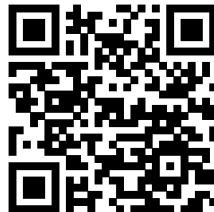
Cat.No. 202 003; Polyclonal rabbit antibody, 50 µg specific antibody (lyophilized)

Data Sheet

Reconstitution/ Storage	50 µg specific antibody, lyophilized. Affinity purified with the immunogen. Albumin and azide were added for stabilization. For reconstitution add 50 µl H ₂ O 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	DOTBLOT: 1 : 1000 up to 1 : 10000 IP: yes ICC: not tested yet IHC: not tested yet IHC_P: not tested yet ELISA: yes suitable for sandwich-ELISA MERIP: yes
Immunogen	N6-methyladenosine fused to BSA
Reactivity	Reacts with: human, rat, mouse, eukaryotes, prokaryotes. Other species not tested yet.
Specificity	Specific for N6-methyladenosine (m6A) with some cross-reactivity to m6Am.

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

Access the online factsheet including applicable protocols at <https://sysy.com/product/202003> or scan the QR-code.



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 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 References for 202 003

- N6-methyladenine DNA Modification in Glioblastoma.
Xie Q, Wu TP, Gimple RC, Li Z, Prager BC, Li Z, Wang P, Wang Y, Gorkin DU, Zhang C, et al. Cell (2018) : . . . **DOTBLOT, IP, ICC, IHC-P; tested species: human**
- Multiplexed profiling facilitates robust m6A quantification at site, gene and sample resolution.
Dierks D, Garcia-Campos MA, Uzonyi A, Safra M, Edelheit S, Rossi A, Sideri T, Varier RA, Brandis A, Stelzer Y, van Werven F, et al. Nature methods (2021) : . . . **IP; tested species: mouse**
- METTL4-mediated nuclear N6-deoxyadenosine methylation promotes metastasis through activating multiple metastasis-inducing targets.
Hsu KW, Lai JC, Chang JS, Peng PH, Huang CH, Lee DY, Tsai YC, Chung CJ, Chang H, Chang CH, Chen JL, et al. Genome biology (2022) 231: 249. . **IP, DOTBLOT, IHC; tested species: human**
- METTL3 enhances dentinogenesis differentiation of dental pulp stem cells via increasing GDF6 and STC1 mRNA stability.
Pan Y, Liu Y, Cui D, Yu S, Zhou Y, Zhou X, Du W, Zheng L, Wan M BMC oral health (2023) 231: 209. . **IP, DOTBLOT, IHC-P; tested species: mouse**
- Downregulation of ALKBH5 rejuvenates aged human mesenchymal stem cells and enhances their therapeutic efficacy in myocardial infarction.
Gao X, Liang X, Liu B, Hong Y, He H, Shen Y, Chen J, Huang X, Hu B, Li W, Li X, et al. FASEB journal : official publication of the Federation of American Societies for Experimental Biology (2023) 3712: e23294. . **IP, DOTBLOT, ICC; tested species: human**
- The enhanced genomic m6A metabolism contributes to the proliferation and migration of TSCC cells.
Xi L, Yang Y, Xu Y, Zhang F, Li J, Liu X, Zhang Z, Du Q International journal of oral science (2022) 141: 11. . **DOTBLOT, ICC, IHC-P; tested species: human**
- Autophagy induction promoted by m6A reader YTHDF3 through translation upregulation of FOXO3 mRNA.
Hao W, Dian M, Zhou Y, Zhong Q, Pang W, Li Z, Zhao Y, Ma J, Lin X, Luo R, Li Y, et al. Nature communications (2022) 131: 5845. . **DOTBLOT, IP, ICC; tested species: mouse**
- DNA 6mA Demethylase ALKBH1 Orchestrates Fatty Acid Metabolism and Suppresses Diet-induced Hepatic Steatosis.
Luo L, Liu Y, Nizigiyimana P, Ye M, Xiao Y, Guo Q, Su T, Luo X, Huang Y, Zhou H Cellular and molecular gastroenterology and hepatology (2022) : . . **DOTBLOT, IHC-P, IP; tested species: mouse**
- Autophagy of the m6A mRNA demethylase FTO is impaired by low-level arsenic exposure to promote tumorigenesis.
Cui YH, Yang S, Wei J, Shea CR, Zhong W, Wang F, Shah P, Kibriya MG, Cui X, Ahsan H, He C, et al. Nature communications (2021) 121: 2183. . **DOTBLOT, IP, IHC-P; tested species: human**
- Validation strategies for antibodies targeting modified ribonucleotides.
Weichmann F, Hett R, Schepers A, Ito-Kureha T, Flatley A, Slama K, Hastert F, Angstmann N, Cardoso CM, König J, Huettelmaier S, et al. RNA (New York, N.Y.) (2020) : . . . **DOTBLOT, IP, ICC; tested species: human**
- N6-methyladenine in DNA antagonizes SATB1 in early development.
Li Z, Zhao S, Nelakanti RV, Lin K, Wu TP, Alderman MH, Guo C, Wang P, Zhang M, Min W, Jiang Z, et al. Nature (2020) : . . **DOTBLOT, IP, ICC; tested species: mouse**
- Methyl Adenine Identification (MadID): High-Resolution Detection of Protein-DNA Interactions.
Umlauf D, Sobbeck M, Crabbe L Methods in molecular biology (Clifton, N.J.) (2020) 2175: 123-138. . **DOTBLOT, IP, ICC; tested species: human**
- N6-methyladenosine demethylases Alkbh5/Fto regulate cerebral ischemia-reperfusion injury.
Xu K, Mo Y, Li D, Yu Q, Wang L, Lin F, Kong C, Balelang MF, Zhang A, Chen S, Dai Q, et al. Therapeutic advances in chronic disease (2020) 11: 2040622320916024. . **DOTBLOT, ICC, IHC-P; tested species: rat**
- N6-Deoxyadenosine Methylation in Mammalian Mitochondrial DNA.
Hao Z, Wu T, Cui X, Zhu P, Tan C, Dou X, Hsu KW, Lin YT, Peng PH, Zhang LS, Gao Y, et al. Molecular cell (2020) : . . . **DOTBLOT, IP, ICC; tested species: human**

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.