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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

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 References for 202 003

N6-methyladenine DNA Modification in Glioblastoma.

Xie Q, Wu TP, Gimple RC, Li Z, Prager BC, Wu Q, Yu Y, 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

Colchicine Blocks Abdominal Aortic Aneurysm Development by Maintaining Vascular Smooth Muscle Cell Homeostasis. Chen M, Yang D, Zhou Y, Yang C, Lin W, Li J, Liu J, Ye J, Huang W, Ma W, Li W, et al.

International journal of biological sciences (2024) 206: 2092-2110. . DOTBLOT, IHC, MERIP; tested species: mouse, human

METTL4-mediated nuclear N6-deoxyadenosine methylation promotes metastasis through activating multiple metastasisinducing 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 Promotes Osteogenic Differentiation of Human Periodontal Ligament Stem Cells through IGF2BP1-Mediated Regulation of Runx2 Stability.

Sun X, Meng X, Piao Y, Dong S, Dong Q

International journal of medical sciences (2024) 214: 664-673. . DOTBLOT, IP, ICC; tested species: human

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

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

The enhanced genomic 6 mA 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

Access the online factsheet including applicable protocols at https://sysy.com/product/202003 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 freezedried) 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 freezethaw cycles.
- Please refer to our tips and hints for subsequent storage of reconstituted antibodies and control peptides and proteins.