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# **METTL3**

Cat.No. 417 003; Polyclonal rabbit antibody, 50 µg specific antibody (lyophilized)

#### **Data Sheet**

Reconstitution/ Storage	50 μg specific antibody, lyophilized. Affinity purified with the immunogen. Albumin was added for stabilization. For <b>reconstitution</b> add 50 μl H <sub>2</sub> 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	WB: 1: 1000 AP-staining IP: not tested yet ICC: 1: 500 IHC: not tested yet IHC-P: not tested yet
Immunogen	Recombinant protein corresponding to AA 2 to 118 from mouse METTL3 (UniProt Id: Q8C3P7)
Reactivity	Reacts with: mouse (Q8C3P7), human (Q86U44), rat (F7FFC6). Other species not tested yet.
Specificity	Specific for isoform 1 K.D. validated PubMed: 34108665

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

#### **Background**

The **METTL3**-METTL14 heterodimer forms a N6-methyltransferase complex that methylates adenosine residues of RNAs at the 5'-[AG]GAC-3' consensus sites. N6-methyladenosine (m6A) has been shown to function as an important regualtory element in many processes like the circadian clock, differentiation of embryonic stem cells, primary miRNA processing, mRNA splicing, translation efficiency, mRNA editing and stability.

#### Selected References for 417 003

RNA m6A modification orchestrates a LINE-1-host interaction that facilitates retrotransposition and contributes to long gene vulnerability.

Xiong F, Wang R, Lee JH, Li S, Chen SF, Liao Z, Hasani LA, Nguyen PT, Zhu X, Krakowiak J, Lee DF, et al. Cell research (2021):.. WB; KD verified; tested species: human

m6A-driven SF3B1 translation control steers splicing to direct genome integrity and leukemogenesis.
Cieśla M, Ngoc PCT, Muthukumar S, Todisco G, Madej M, Fritz H, Dimitriou M, Incarnato D, Hellström-Lindberg E, Bellodi C
Molecular cell (2023) 837: 1165-1179.e11.. WB; KD verified; tested species: human

Enhancer RNA m6A methylation facilitates transcriptional condensate formation and gene activation. Lee JH, Wang R, Xiong F, Krakowiak J, Liao Z, Nguyen PT, Moroz-Omori EV, Shao J, Zhu X, Bolt MJ, Wu H, et al. Molecular cell (2021) 8116: 3368-3385.e9. . WB; tested species: human

#### **Selected General References**

m(6)A RNA modification controls cell fate transition in mammalian embryonic stem cells. Batista PJ et al. Cell Stem Cell (2014) PubMed:25456834

The m(6)A Methyltransferase METTL3 Promotes Translation in Human Cancer Cells. Lin S et al. Mol. Cell (2016) PubMed:27117702

Structural basis of N(6)-adenosine methylation by the METTL3-METTL14 complex. Wang X et al. Nature (2016) PubMed:27281194

N6-methyladenosine modification destabilizes developmental regulators in embryonic stem cells. Wang Y et al. Nat. Cell Biol. (2014) PubMed:24394384

A METTL14 complex mediates mammalian nuclear RNA N6-adenosine methylation. Liu J et al. Nat. Chem. Biol. (2014) PubMed:24316715

RNA-methylation-dependent RNA processing controls the speed of the circadian clock. Fustin JM et al. Cell (2013) PubMed:24209618

Access the online factsheet including applicable protocols at <a href="https://sysy.com/product/417003">https://sysy.com/product/417003</a> 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.