For Research Use Only

m6A Monoclonal antibody, PBS Only

Catalog Number: 68055-1-PBS



Basic Information

Catalog Number:

68055-1-PBS

Size:

1000 $\,\mu$ g/ml Source:

Mouse Isotype: lgG3

GenBank Accession Number:

m6A

GeneID (NCBI):

Full Name:

Purification Method: Protein A purification

CloneNo.: 1D5E10

Applications

Tested Applications:

IHC, RIP, Dot Blot, ELISA, Indirect ELISA

Species Specificity: chemical compound, m6a

Background Information

 $m6A \ (N6-methyladenosine) \ is \ the \ most \ abundant \ internal \ modification \ in \ mammalian \ mRNA. \ This \ modification \ is \ modification \ in \ mammalian \ mRNA.$ installed by the m6A methyltransferases or termed "writers" such as METTL3 and METTL14, and can be reversed by demethylases that serve as "erasers" such as FTO and ALKBH5. The stability of m6A-modified mRNA is regulated by $m6A\ reader\ protein\ YTHDFs, which\ recognizes\ m6A\ and\ reduces\ the\ stability\ of\ target\ transcripts.\ m6A\ modification$ and its regulatory proteins play critical roles in cancer pathogenesis and progression. m6A modification is also invovled in viruses life cycles, suggesting that drugs targets to m6A pathway could be used for antiviral thereapy.

Protocol for Dot Blot:

https://www.ptglab.com/protocol/68055-1-lgDotBlot.pdf

Storage

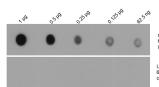
Storage:

Store at -80°C.

The product is shipped with ice packs. Upon receipt, store it immediately at -80°C

PBS Only

Selected Validation Data



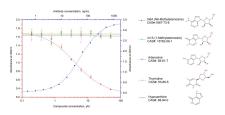
Total RNA was isolated from HEK-293 cell line and was dotted to NC membrane at different

amount as indicated above the dots. The membrane was blocked with BSA and blotted with m6A antibody 68055-1-lg at 1:2000 followed by incubation of HRP-goat antimuse secondary antibody. Signal was developed by ECL substrate. A parallel dot

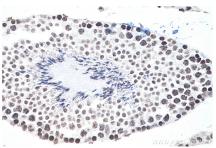
blot was performed using unrelated antibody with the same isotype (UCP2 antibody 66700-1-lg) at the same dose. This data was

m6A antibody 68055-1-lg (Isotype IgG3)

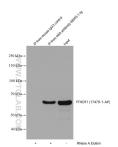
UCP2 antibody 66700-1-lg (negative

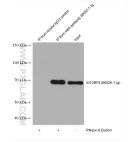


Indirect ELISA and competitive ELISA results show that this antibody is specific to m6A. Indirect ELISA was performed by coating BSA conjugated m6A at 20ng/well followed by blocking with 196 BSA. Serial diluted primary antibody was added to the plates and incubated at 37°C. HRP-goat anti-mouse was used for detection. Competitive ELISA was performed similarly except that different concentration of m6A or its structure analogue compounds are mixed in primary antibody



Immunohistochemical analysis of paraffinembedded mouse testis tissue slide using 68055-1-Ig (m6A antibody) at dilution of 1:4000 (under 40x lens). Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0). This data was developed using the same antibody clone with 68055-1-PBS in a different storage buffer formulation.





HEK-293 cells were lysised and immunoprecipitated with Protein A-m6A antibody and Protein A-mouse IgG3 control antibody respectively in the presence of RNAase inhibotor cocktail. The immunoprecipitated complex was washed diggested by RNAse A followed by western blot with YTHDF1(m6A reader) antibody 17479-1-AP (1:2000). (Lysate: 3.6mg per IP; IP: 15µg antibody and 50µL beads, 4 hours at 4°C; Diggestion: 50µg/mL * 80µL RNAse A for 1

HEK-293 cells were lysised and immunoprecipitated with Protein A-m6A antibody and Protein A-mouse IgG3 control antibody respectively in the presence of RNAase inhibotor cocktail. The immunoprecipitated complex was washed diggested by RNAse A followed by western blot with IGF2BP3 (m6A reader) antibody 66526-1-Ig (1:2000). (Lysate: 4.0 mg per IP; IP: 30µg antibody and 50µL beads, 4 hours at 4°C; Diggestion: 50µg/mL * 80µL RNAse A for 1