## For Research Use Only

# m6A Monoclonal antibody

Catalog Number: 68055-1-lg 71 Publications



**Basic Information** 

Catalog Number: 68055-1-lg

Concentration: 1000 ug/ml

Source: Mouse Isotype: IgG3 GenBank Accession Number:

m6A

GeneID (NCBI):

Full Name:

Purification Method:

Protein A purification

CloneNo.: 1D5E10

Recommended Dilutions:

RIP 1:1000-1:4000 IHC 1:2000-1:8000 ELISA 1:2000-1:20000 Dot Blot 1:1000-1:4000

**Applications** 

**Tested Applications:** 

IHC, RIP, Dot Blot, ELISA

Cited Applications:

WB, IHC, IF, IP, RIP, ELISA

Species Specificity:

chemical compound, m6a

Cited Species:

human, mouse, rat, pig, monkey

Note-IHC: suggested antigen retrieval with TE buffer pH 9.0; (\*) Alternatively, antigen retrieval may be performed with citrate

buffer pH 6.0

**Positive Controls:** 

RIP: RNA,

IHC: mouse testis tissue, human lung cancer tissue, human breast cancer tissue, human colon cancer tissue, rat lymph node

ELISA: m6A,

Dot Blot: RNA,

## **Background Information**

m6A (N6-methyladenosine) is the most abundant internal modification in mammalian mRNA. This modification is 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

### **Notable Publications**

Author	Pubmed ID	Journal	Application
Lee J Martin	36359844	Cells	IHC
Meige Sun	36358640	Cancers (Basel)	
Shu Fang	35571033	Front Genet	

Storage

Storage:

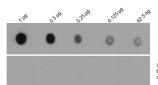
Store at -20°C. Stable for one year after shipment.

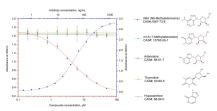
Storage Buffer:

PBS with 0.02% sodium azide and 50% glycerol, pH7.3

Aliquoting is unnecessary for -20°C storage

### Selected Validation Data



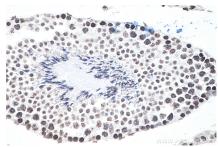


Indirect ELISA and competitive ELISA results

show that this antibody is specific to m6A. Indirect ELISA was performed by coating BSA conjugated m6A at 20ng/well followed by blocking with 1% BSA. Serial diluted primary antibody was added to the plates and

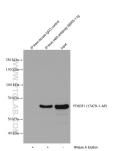
compounds are mixed in primary antibody

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



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).

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 68055-1-1g at 1:2000 followed by incubation of HRP-goat anti-mouse 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.



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 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