

Recombinant YTHDF2 protein

Catalog No: 31573, 31973

Lot No: 30616001

Expressed In: Baculovirus

Quantity: 20, 1000 µg

Concentration: 0.3 µg/µl

Source: Human

Buffer Contents: Recombinant YTHDF2 protein is supplied at a concentration of 0.3 µg/µl in 25 mM HEPES pH 7.5, 300 mM NaCl, 5% glycerol, 0.04% Triton X-100, 0.2 mM TCEP.

Background: N6-methylated adenine (m6A) is prevalently present in nearly all RNA types and can be found in all organisms from bacteria to humans. It preferentially appears around stop codons and within long internal exons in mammalian messenger RNAs. m6A plays an important role in the efficiency of mRNA splicing, processing, translation efficiency, editing and mRNA stability. m6A is also found in other RNA molecules, such as primary miRNA (pri-miRNAs).

YTHDF2 (YT521-B homology domain family 2, also known as CAHL, HGRG8, NY-REN-2) is a member of the YTH (YT521-B homology) superfamily containing YTH domain. Human YTH domain family proteins include three members, YTHDF1-3, which mainly localized in the cytoplasm. YTHDF proteins contain a highly conserved single-stranded RNA-binding domain which can specifically recognize and bind m6A-containing RNAs. An important role of YTHDF2 is in regulating mRNA stability. YTHDF2 recognizes many m6A-containing mRNAs that are not being actively translated and recruits them to cytoplasmic processing (P) bodies for degradation. YTHDF2 preserves 5'UTR methylation of stress-induced transcripts and promotes cap-independent mRNA translation by competitive binding and limiting the m6A 'eraser' FTO from demethylation.

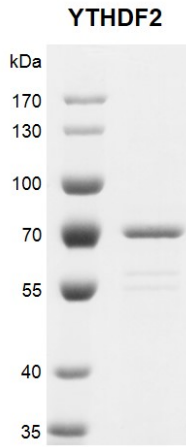
Protein Details: Recombinant YTHDF2 protein was expressed in a Baculovirus expression system as the full length protein (accession number NP_057342.2) with an N-terminal FLAG-Tag. The molecular weight of YTHDF2 is 63.6 kDa.

Application Notes: Recombinant YTHDF2 protein is suitable for use in protein-protein interaction and RNA binding assays.

HTRF Assay: 3 µM m6A ssDNA oligos (5'-GTTGG/ m6A/CTT-3') were incubated with YTHDF2 protein in binding buffer including 50 mM HEPES-NaOH pH 7.0, 0.1% BSA for 1 hour at RT. Anti-FLAG antibody was used to detect reaction products.

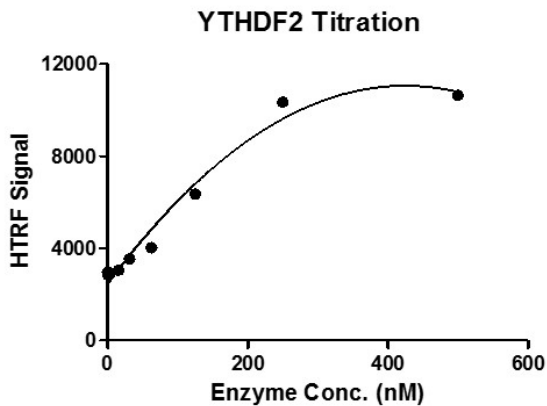
Binding Assay: 45 pmol biotinylated m6A ssDNA oligos (DNA sequence: 5'-GTTGCCTGTTCTGTTGG/m6A/CTTGCCTGT-3') were incubated with YTHDF2 protein in binding buffer including 25 mM Tris-HCl pH 7.4, 150 mM NaCl, 5% glycerol, 0.1% Triton X-100 for 3 hr at 4C. DNA-YTHDF2 compounds were incubated with streptavidin beads for 1 hr at 4C and pull-down products were run on a 10% SDS-PAGE gel, stained with Coomassie Blue. Biotinylated, unmethylated ssDNA oligos were used as negative control. YTHDF2 preferentially binds to ssDNA with m6A modification compared with unmodified ssDNA.

Storage and Guarantee: Recombinant proteins in solution are temperature sensitive and must be stored at -80°C to prevent degradation. Avoid repeated freeze/thaw cycles and keep on ice when not in storage. This product is for research use only and is not for use in diagnostic procedures. This product is guaranteed for 6 months from date of arrival.



Recombinant YTHDF2 protein gel.

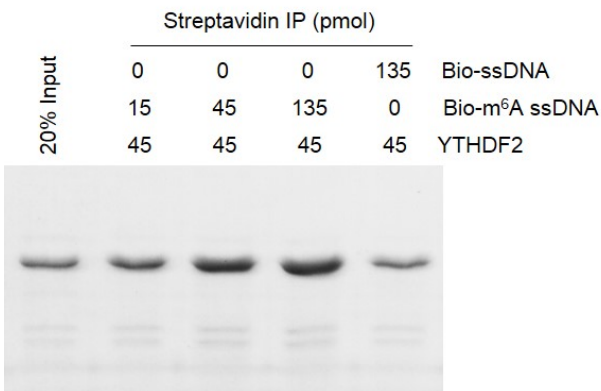
Recombinant YTHDF2 protein was run on an 8% SDS-PAGE gel and stained with Coomassie Blue.



HTRF Assay for Recombinant YTHDF2 protein activity.

3 μ M m6A ssDNA oligos (5'-GTTGG/ m6A/CTT-3') were incubated with YTHDF2 protein in binding buffer for 1 hour at room temperature.

Anti-FLAG antibody was used to detect reaction products.



Binding Assay for Recombinant YTHDF2 protein activity.

45 pmol biotinylated m6A ssDNA oligos (DNA sequence: 5'-GTTGCCTGTTTCGTGTTGG/m6A/ CTTGCCTGT-3') were incubated with YTHDF2 protein in binding buffer including 25 mM Tris-HCl pH 7.4, 150 mM NaCl, 5% glycerol, 0.1% Triton X-100 for 3 hr at 4C. DNA-YTHDF2 compounds were incubated with streptavidin beads for 1 hr at 4C and pull-down products were run on a 10% SDS-PAGE gel, stained with Coomassie Blue. Biotinylated, unmethylated ssDNA oligos were used as negative control. YTHDF2 preferentially binds to ssDNA with m6A modification compared with unmodified ssDNA.