abcam

ab5828

Anti-Y14 antibody [4C4]

Key facts

Isotype	IgG2b
Host species	Mouse
Storage buffer	Preservative: 0.1% Sodium azide Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	Native Full Length Protein corresponding to Human RBM8A. Database link Q9Y5S9
Clone number	4C4
Purification technique	Affinity purification Protein A
Concentration	1 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →
Purification notes	Purified from tissue culture supernatant.

Reactivity data

IHC-P

Tested

Species	Human
Dilution info	1 µg/mL
Notes	Perform heat-mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

ICC

Tested

Species	Human
Dilution info	-
Notes	-

Flow Cyt

Tested

Species	Human
Dilution info	1-2 µg for 10^6 Cells
Notes	ab170192 - Mouse monoclonal IgG2b, is suitable for use as an isotype control with this antibody.

WB

Tested

Species	Human
Dilution info	-
Notes	-

Target data

See full target information RBM8A

Function

Required for pre-mRNA splicing as component of the spliceosome (PubMed:28502770, PubMed:29301961). Core component of the splicing-dependent multiprotein exon junction complex (EJC) deposited at splice junctions on mRNAs. The EJC is a dynamic structure consisting of core proteins and several peripheral nuclear and cytoplasmic associated factors that join the complex only transiently either during EJC assembly or during subsequent mRNA metabolism. The EJC marks the position of the exon-exon junction in the mature mRNA for the gene expression machinery and the core components remain bound to spliced mRNAs throughout all stages of mRNA metabolism thereby influencing downstream processes including nuclear mRNA export, subcellular mRNA localization, translation efficiency and nonsense-mediated mRNA decay (NMD). The MAGOH-RBM8A heterodimer inhibits the ATPase activity of EIF4A3, thereby trapping the ATP-bound EJC core onto spliced mRNA in a stable conformation. The

MAGOH-RBM8A heterodimer interacts with the EJC key regulator PYM1 leading to EJC disassembly in the cytoplasm and translation enhancement of EJC-bearing spliced mRNAs by recruiting them to the ribosomal 48S preinitiation complex. Its removal from cytoplasmic mRNAs requires translation initiation from EJC-bearing spliced mRNAs. Associates preferentially with mRNAs produced by splicing. Does not interact with pre-mRNAs, introns, or mRNAs produced from intronless cDNAs. Associates with both nuclear mRNAs and newly exported cytoplasmic mRNAs. The MAGOH-RBM8A heterodimer is a component of the nonsense mediated decay (NMD) pathway. Involved in the splicing modulation of BCL2L1/Bcl-X (and probably other apoptotic genes); specifically inhibits formation of proapoptotic isoforms such as Bcl-X(S); the function is different from the established EJC assembly.

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	Y14 also known as RBM8A is a protein integral to the exon junction complex (EJC) with an approximate mass of 18 kDa. It localizes mainly in the nucleus of cells. Y14 works with other proteins such as MAGOH serving a role in post-splicing mRNA regulation. Across various tissues Y14 shows expression but is particularly notable in tissues with high levels of mRNA processing like the brain and testis.
Biological function summary	Y14 functions in mRNA surveillance and quality control mechanisms. It forms part of the exon junction complex a multi-protein structure deposited on mRNA after splicing occurs. The component acts importantly in nonsense-mediated mRNA decay (NMD) a process that ensures defective mRNAs do not translate into dysfunctional proteins. Y14 contributes significantly to mRNA export from the nucleus binding to spliced mRNA and guiding them towards translation sites in the cytoplasm.
Pathways	Y14 plays a significant role in the nonsense-mediated mRNA decay pathway working in tandem with proteins such as UPF1 and UPF3. This pathway helps

	identify and degrade mRNA transcripts with premature stop codons therefore protecting cells from expressing truncated proteins that may disrupt normal cellular functions. Moreover Y14 is linked to the mRNA export pathway ensuring that only properly spliced messages reach the ribosome for translation.
Associated diseases and disorders	Y14 relevance appears in relation to neurodevelopmental disorders and certain cancers. Mutations or dysregulation of Y14 might contribute to conditions such as intellectual disability due to its involvement in mRNA surveillance that affects neuronal development. Additionally aberrant expression of Y14 has connections to breast cancer where it interacts with proteins involved in altered signaling pathways impacting cancer progression and metastasis.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

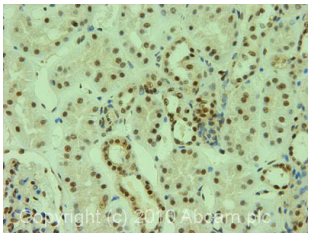
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

4 product images

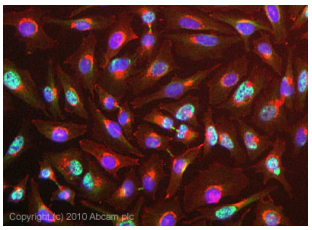


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Y14 antibody [4C4] (ab5828)

Y14 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using mouse Anti-Y14 antibody

IHC image of ab5828 staining in human kidney formalin fixed paraffin embedded tissue section, performed on a Leica Bond™ system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab5828, 1µg/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

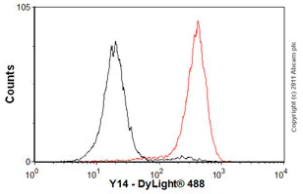
For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.



Immunocytochemistry - Anti-Y14 antibody [4C4] (ab5828)

Y14 Immunocytochemistry staining using mouse Anti-Y14 antibody

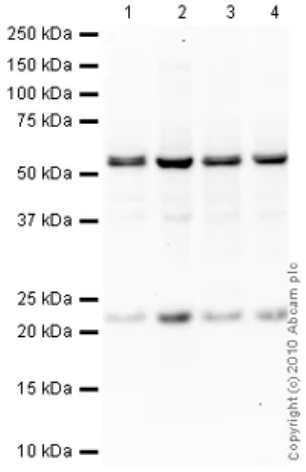
ICC/IF image of ab5828 stained HeLa cells. The cells were 4% formaldehyde fixed (10 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab5828, 5µg/ml) overnight at +4°C. The secondary antibody (green) was Alexa Fluor® 488 goat anti-mouse IgG (H+L) used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.



Flow Cytometry - Anti-Y14 antibody [4C4] (ab5828)

Y14 Flow Cytometry staining using mouse Anti-Y14 antibody

Overlay histogram showing HeLa cells stained with ab5828 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab5828, 1µg/1x10^6 cells) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (ab96879) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG2b [PLPV219] (ab91366, 2µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a positive signal in HeLa cells fixed with 4% paraformaldehyde (10 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.



Western blot - Anti-Y14 antibody [4C4] (ab5828)

Y14 Western blot staining using mouse Anti-Y14 antibody

All lanes:
Western blot - Anti-Y14 antibody [4C4] (ab5828) at 1 µg/mL

Lane 1:
HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate at 10 µg

Lane 2:
Jurkat (Human T cell lymphoblast-like cell line) Whole Cell Lysate at 10 µg

Lane 3:
HepG2 (Human hepatocellular liver carcinoma cell line) Whole Cell Lysate at 10 µg

Lane 4:
HEK293 (Human embryonic kidney cell line) Whole Cell Lysate at 10 µg

Secondary

All lanes:
Western blot - Goat Anti-Mouse IgG H&L (HRP) preadsorbed (ab97040) at 1/5000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 19 kDa

Observed band size: 21 kDa, 53 kDa

Exposure time: 8min

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.