abcam

Product datasheet

Anti-Y14 antibody [4C4] ab5828

★★★★★ 7 Abreviews 6 References 4 Images

Overview

Product name	Anti-Y14 antibody [4C4]
Description	Mouse monoclonal [4C4] to Y14
Host species	Mouse
Tested applications	**Suitable for:** Flow Cyt, WB, ICC, IHC-P
Species reactivity	**Reacts with:** Human
Immunogen	Full length native protein (purified) (Human).
General notes	The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -80°C. Avoid freeze / thaw cycle.
Storage buffer	Preservative: 0.1% Sodium azide Constituent: PBS
Purity	Protein A purified
Purification notes	Purified from tissue culture supernatant.
Clonality	Monoclonal
Clone number	4C4
Myeloma	Sp2/0
Isotype	IgG2b

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab5828 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt		Use 1-2µg for 10^6 cells. **ab170192** - Mouse monoclonal IgG2b, is suitable for use as an isotype control with this antibody.
WB	★★★★☆ (2)	Use at an assay dependent concentration. Predicted molecular weight: 19 kDa.
ICC	★★★★★ (1)	Use at an assay dependent concentration.
IHC-P	★★★★★ (2)	Use a concentration of 1 µg/ml. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

Target

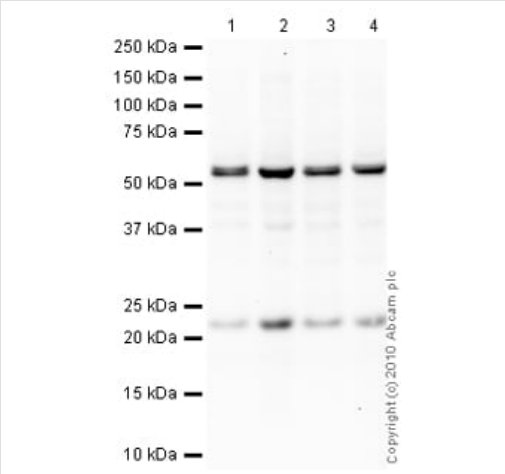
Function Component of a splicing-dependent multiprotein exon junction complex (EJC) deposited at splice junction on mRNAs. The EJC is a dynamic structure consisting of a few core proteins and several more peripheral nuclear and cytoplasmic associated factors that join the complex only transiently either during EJC assembly or during subsequent mRNA metabolism. Core components of the EJC, that remains bound to spliced mRNAs throughout all stages of mRNA metabolism, functions to mark the position of the exon-exon junction in the mature mRNA and thereby influences downstream processes of gene expression including mRNA splicing, nuclear mRNA export, subcellular mRNA localization, translation efficiency and nonsense-mediated mRNA decay (NMD). The heterodimer MAGOH-RBM8A interacts with PYM that function to enhance the translation of EJC-bearing spliced mRNAs by recruiting them to the ribosomal 48S preinitiation complex. Remains associated with mRNAs in the cytoplasm until the mRNAs engage the translation machinery. 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. Complex with MAGOH is a component of the nonsense mediated decay (NMD) pathway.

Tissue specificity Ubiquitous.

Sequence similarities Belongs to the RBM8A family.
Contains 1 RRM (RNA recognition motif) domain.

Cellular localization Nucleus. Nucleus speckle. Cytoplasm. Nucleocytoplasmic shuttling protein. Travels to the cytoplasm as part of the exon junction complex (EJC) bound to mRNA. Colocalizes with the core EJC, THOC4, NXF1 and UAP56 in the nucleus and nuclear speckles.

Images



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

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

Lane 1 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate
Lane 2 : Jurkat (Human T cell lymphoblast-like cell line) Whole Cell Lysate
Lane 3 : HepG2 (Human hepatocellular liver carcinoma cell line) Whole Cell Lysate
Lane 4 : HEK293 (Human embryonic kidney cell line) Whole Cell Lysate

Lysates/proteins at 10 µg per lane.

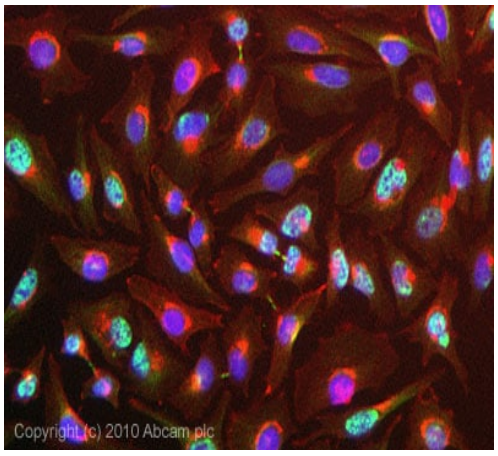
Secondary
All lanes : Goat Anti-Mouse IgG H&L (HRP) preadsorbed (**ab97040**) at 1/5000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

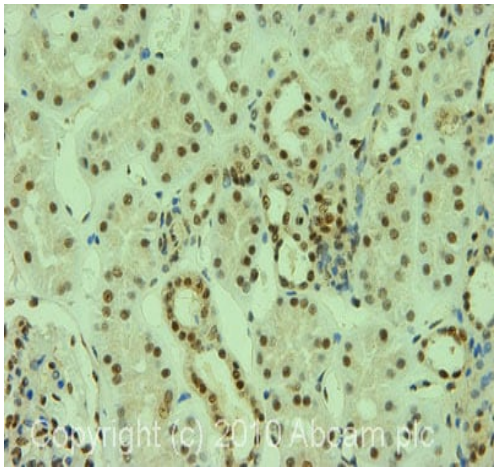
Predicted band size: 19 kDa
Observed band size: 21 kDa
Additional bands at: 53 kDa. We are unsure as to the identity of these extra bands.

Exposure time: 8 minutes



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

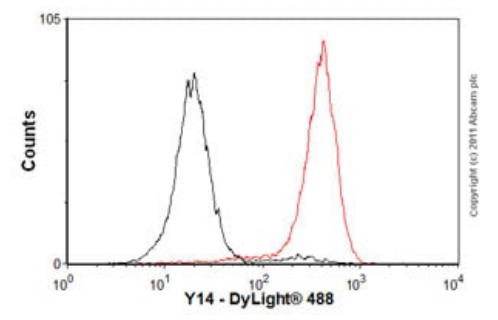
ICC/IF image of ab5828 stained HeLa cells. The cells were 4% formaldehyde fixed (10 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (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.



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

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.



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

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 22ºC. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (**ab96879**) at 1/500 dilution for 30 min at 22ºC. 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.

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