abcam

Product datasheet

Anti-Myc tag antibody [9E10] ab32

★★★★★ 29 Abreviews 403 References 6 Images

Overview

Product name	Anti-Myc tag antibody [9E10]
Description	Mouse monoclonal [9E10] to Myc tag
Host species	Mouse
Tested applications	**Suitable for:** ICC/IF, Flow Cyt, WB, IP, ELISA, IHC-Fr, Purification
Species reactivity	**Reacts with:** Species independent
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	Myc tagged proteins and myc tag expressing cells.
General notes	If you require this antibody in a particular buffer formulation or a particular conjugate for your experiments, please contact **orders@abcam.com** or you can find further information **here**.

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	pH: 7.40 Preservative: 0.02% Sodium azide Constituents: PBS, 6.97% L-Arginine
Purity	Protein G purified
Clonality	Monoclonal
Clone number	9E10
Myeloma	Sp2/0
Isotype	IgG1

Light chain type kappa

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab32 in the following tested applications.

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

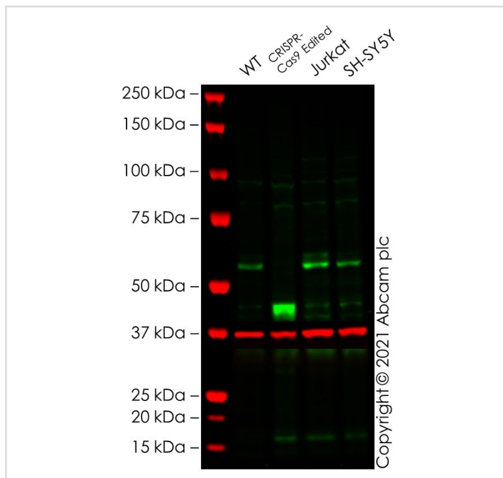
Application	Abreviews	Notes
ICC/IF	★★★★★ (6)	Use a concentration of 5 µg/ml.
Flow Cyt		Use 1µg for 10^6 cells. **ab170190** - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.
WB	★★★★★ (13)	1/500 - 1/1000.
IP	★★★★★ (3)	Use at 6 µg/mg of lysate.
ELISA		Use at an assay dependent concentration.
IHC-Fr		1/1000. See Abreviews.
Purification		Use at an assay dependent concentration.

Target

Relevance Epitope tags are short peptide sequences that are easily recognized by tag-specific antibodies. Due to their small size, epitope tags do not affect the tagged protein's biochemical properties. Most often sequences encoding the epitope tag are included with target DNA at the time of cloning to produce fusion proteins containing the epitope tag sequence. This allows anti-epitope tag antibodies to serve as universal detection reagents for any tag containing protein produced by recombinant means. This means that anti-epitope tag antibodies are a useful alternative to generating specific antibodies to identify, immunoprecipitate or immunoaffinity purify a recombinant protein. The anti-epitope tag antibody is usually functional in a variety of antibody-dependent experimental procedures. Expression vectors producing epitope tag fusion proteins are available for a variety of host expression systems including bacteria, yeast, insect and mammalian cells.

Cellular localization Nuclear

Images



Western blot - Anti-Myc tag antibody [9E10] (ab32)

All lanes : Anti-Myc tag antibody [9E10] (ab32) at 1/200 dilution

Lane 1 : Wild-type HEK-293T cell lysate

Lane 2 : MYC CRISPR-Cas9 edited HEK-293T cell lysate

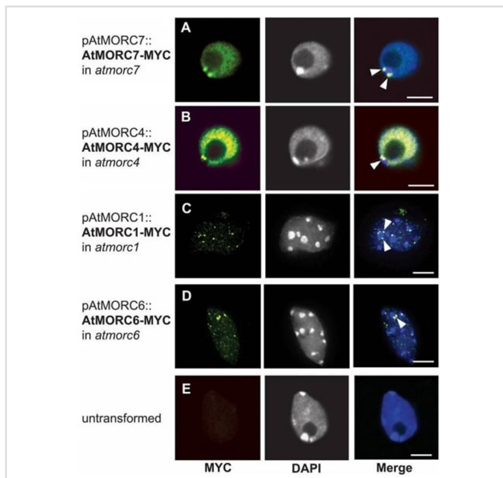
Lane 3 : Jurkat cell lysate

Lane 4 : SH-SY5Y cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

False colour image of Western blot: Anti-Myc tag antibody [9E10] staining at 1/200 dilution, shown in green; Rabbit Anti-GAPDH antibody [EPR16891] (**ab181602**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab32 was shown to bind specifically to Myc tag. A band was observed at 57 kDa in wild-type HEK-293T cell lysates with no signal observed at this size in MYC CRISPR-Cas9 edited cell line **ab256500** (CRISPR-Cas9 edited cell lysate **ab263850**). The band observed in the CRISPR-Cas9 edited lysate lane below 57 kDa is likely to represent a truncated form of Myc tag. This has not been investigated further and the functional properties of the gene product have not been determined. To generate this image, wild-type and MYC CRISPR-Cas9 edited HEK-293T cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Mouse IgG H&L (IRDye® 800CW) preabsorbed (**ab216772**) and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed (**ab216777**) at 1/20000 dilution.

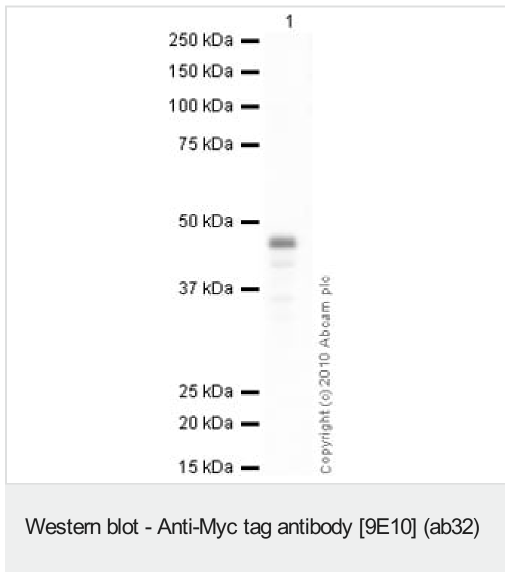


Immunocytochemistry/ Immunofluorescence - Anti-Myc tag antibody [9E10] (ab32)

Image from Harris CJ et al., PLoS Genet. 2016;12(5):e1005998. Fig 5.; doi: 10.1371/journal.pgen.1005998. Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/4.0/

(A-D) Representative examples of body forming AtMORC7-MYC, AtMORC4-MYC, At-MORC1-MYC, and AtMORC6-MYC nuclei, respectively. (E) Untransformed wt nucleus subjected to the same antibody staining and imaging procedure. Left panels = anti-MYC channel; middle panels = DAPI channel (gray scaled). DAPI stains DNA, defining the position of dense chromocenters as high intensity white foci; right panels = merged channels (DAPI in blue, MYC in green). White triangles indicate examples of chromocenter adjacent AtMORC localization. Scale bars = 5 µM.

Leaves from three-week old plants were fixed in 4% paraformaldehyde in TRIS buffer (10 mM TRIS pH 7.5, 10 mM EDTA, and 100 mM NaCl) for 20 minutes and washed twice in TRIS buffer. Leaves were chopped in 200–400 microliters lysis buffer (15 mM TRIS pH 7.5, 2 mM EDTA, 0.5 mM spermine, 80 mM KCl, 20 mM NaCl, and 0.1% Triton X-100) and filtered through a 3 µM cell strainer. 5 µL of nuclei suspension was added to 12 µL of sorting buffer (100mM TRIS pH 7.5, 50mM KCl, 2mM MgCl2, 0.05% Tween-20, and 20.5% sucrose) and air dried on chloroform dipped microscope slides for two hours and then post-fixed in 4% paraformaldehyde in PBS for 20 minutes. Slides were washed three times in PBS and incubated in blocking buffer (3% BSA, and 10% horse serum in PBS) for 30 minutes at 37°C. Nuclei were incubated at 4°C overnight in mouse monoclonal antibody against c-Myc (9E10, ab32; 1/200). Slides were washed in PBS and incubated with goat anti-mouse FITC antibody (**ab7064**; 1/200) for 90 minutes at room temperature. Following PBS washes, nuclei were counterstained and mounted in Vectashield mounting media with DAPI. Nuclei were analyzed with a Zeiss LSM 710 Confocal microscope at 63X or 100X magnification using Zen software.



Western blot - Anti-Myc tag antibody [9E10] (ab32)

Anti-Myc tag antibody [9E10] (ab32) at 1 µg/ml + E. coli Positive Control (Escherichia coli) Whole Cell Lysate (**ab5395**) at 10 µg

Secondary
Goat polyclonal to Mouse IgG - H&L - Pre-Adsorbed (HRP) at 1/3000 dilution

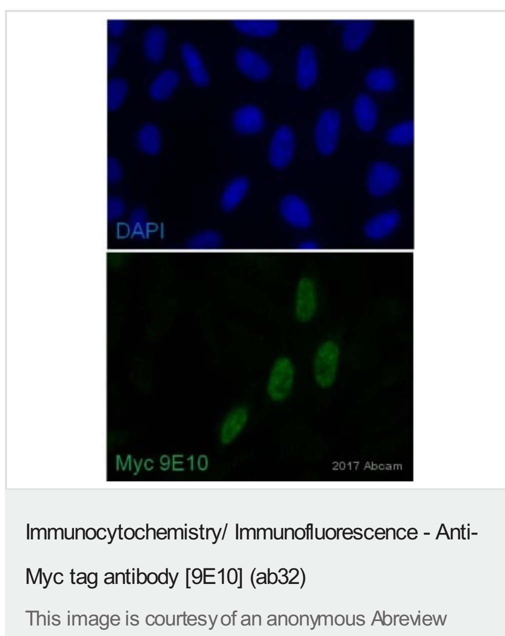
Developed using the ECL technique.

Performed under reducing conditions.

Observed band size: 45 kDa

Exposure time: 1 minute

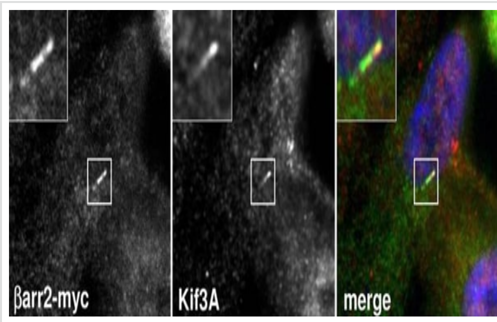
Lysate from E. coli recombinantly expressing 11 commonly used tags including myc tag.



Immunocytochemistry/ Immunofluorescence - Anti-Myc tag antibody [9E10] (ab32)

This image is courtesy of an anonymous Abreview

Ab32 staining a Myc tagged protein in HeLa cells by ICC/IF (Immunocytochemistry/Immunofluorescence). Endogenous c-myc was not detected under these conditions. Cells were fixed with paraformaldehyde, permeabilized with 0.5% Triton and blocked with 5% Serum for 30 minutes at 25°C. Samples were incubated with primary antibody (1/1000 in 5% Serum) for 1 hour at 25°C. An Alexa Fluor® 488 conjugated Goat anti-Mouse was used as a secondary antibody.

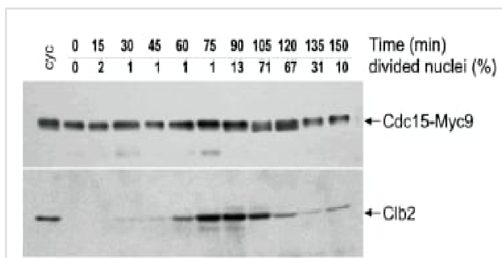


Immunocytochemistry/ Immunofluorescence - Anti-Myc tag antibody [9E10] (ab32)

Image from Molla-Herman A et al., PLoS One. 2008;3(11):e3728. Fig 8(B).; doi: 10.1371/journal.pone.0003728. Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/4.0/

RPE1 cells grown on coverslips were transfected with βarr2-myc, grown in low serum and then fixed and stained for Kif3A (red) and ab32 (green). Insets show higher magnifications of a representative PC. Kif3A was found in the cytoplasm and at the tip of the axoneme where it was colocalized with βarr2.

Cells were incubated with primary antibodies in permeabilization buffer (PBS with 1 mg/mL bovine serum albumin (PBS-BSA) and 0.1% triton-X-100) for 45 minutes at room temperature. After two washes with PBS-BSA, cells were incubated for 30 minutes at room temperature in PBS-BSA containing secondary antibodies. After one wash with PBS-BSA and two washes in PBS, cells were laid down on microscope slides in a PBS–glycerol mix (50/50) with DAPI.



Western blot - Anti-Myc tag antibody [9E10] (ab32)

Menssen R et al., (2001) Curr Biol. Mar 6;11(5):345-50.

Phosphorylation of Cdc15 changes during the cell cycle. Exponentially growing cells (cyc) of CDC15-MYC9 (W1114) were arrested in G1 with a factor pheromone and released into fresh medium at 25°C. Cells were harvested at the indicated times, the percentage of divided nuclei was determined by DAPI staining of fixed cells, and proteins were analyzed by western blotting with 9E10 (ab32).

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