abcam

Product datasheet

Anti-c-Myc antibody [Y69] - ChIP Grade - BSA and Azide free ab168727





★★★★★ 11 Abreviews 5 References 35 Images

Overview

Product name Anti-c-Myc antibody [Y69] - ChIP Grade - BSA and Azide free

Description Rabbit monoclonal [Y69] to c-Myc - BSA and Azide free

Host species Rabbit

Specificity This antibody is specific for endogenous c-Myc. It does not detect Myc tag. Expression levels of

the target protein vary with sample type and some optimization may be required.

FURTHER INFORMATION ON POSITIVE CONTROLS (Chinese version)

Tested applications Suitable for: Flow Cyt (Intra), WB, ICC/IF, ChIP-sequencing, IP, IHC-P, ChIC/CUT&RUN-seq

Species reactivity Reacts with: Mouse, Rat, Human

Immunogen Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: Jurkat, HeLa, HEK-293T, Raji, MCF-7, K562, A20, AR42J, Rat-1, rat pancreas, Neuro-2a

> cell lysates, L363 MM and CA46 cells. ICC/IF: HEK293 and HeLa cells. IHC-P: Human Burkitt lymphoma, diffuse large B cell lymphoma, adenocarcinoma of the colon, lung adenocarcinoma, gastric adenocarcinoma, urinary bladder transitional carcinoma, esophagus, glioblastoma and low-grade glioma tumor tissues, human skin tissue. IP:Jurkat cell lysate. Flow Cyt (intra), ChIP-

seq, ChlC/C&R-seq: HeLa cells, HEK293 cells.

General notes ab168727 is the carrier-free version of ab32072.

> Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency.

This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-

based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

This product is compatible with the Maxpar[®] Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar[®] is a trademark of Fluidigm Canada Inc.

This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free production

For more information **see here**.

Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb**[®] **patents**.

Properties

Form Liquid

Storage instructions Shipped at 4°C. Store at +4°C. Do Not Freeze.

Storage buffer Constituent: PBS

Carrier free Yes

Purity Protein A purified

Clonality Monoclonal

Clone number Y69
Isotype IgG

Applications

The Abpromise guarantee

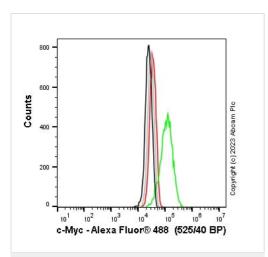
Our **Abpromise guarantee** covers the use of ab168727 in the following tested applications.

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

Application	Abreviews	Notes
Flow Cyt (Intra)		Use at an assay dependent concentration. Please refer to the original abID, <u>ab32072</u> , for information on recommended dilutions.
WB	★★★★ (5)	Use at an assay dependent concentration. Detects a band of approximately 57 kDa (predicted molecular weight: 48 kDa). Please refer to the original ablD, <u>ab32072</u> , for information on recommended dilutions.
ICC/IF	*** <u>*</u> (2)	Use at an assay dependent concentration. Please refer to the original abID, ab32072 , for information on recommended dilutions.
ChIP-sequencing		Use at an assay dependent concentration.
IP	****(2)	Use at an assay dependent concentration. Please refer to the original abID, ab32072 , for information on recommended dilutions.

Application	Abreviews	Notes
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. Please refer to the original abID, <u>ab32072</u> , for information on recommended dilutions.
ChIC/CUT&RUN-seq		Use at an assay dependent concentration.

Target	
Function	Participates in the regulation of gene transcription. Binds DNA in a non-specific manner, yet also specifically recognizes the core sequence 5'-CAC[GA]TG-3'. Seems to activate the transcription of growth-related genes.
Involvement in disease	Note=Overexpression of MYC is implicated in the etiology of a variety of hematopoietic tumors. Note=A chromosomal aberration involving MYC may be a cause of a form of B-cell chronic lymphocytic leukemia. Translocation t(8;12)(q24;q22) with BTG1. Defects in MYC are a cause of Burkitt lymphoma (BL) [MIM:113970]. A form of undifferentiated malignant lymphoma commonly manifested as a large osteolytic lesion in the jaw or as an abdominal mass. Note=Chromosomal aberrations involving MYC are usually found in Burkitt lymphoma. Translocations t(8;14), t(8;22) or t(2;8) which juxtapose MYC to one of the heavy or light chain immunoglobulin gene loci.
Sequence similarities	Contains 1 basic helix-loop-helix (bHLH) domain.
Post-translational modifications	Phosphorylated by PRKDC. Phosphorylation at Thr-58 and Ser-62 by GSK3 is required for ubiquitination and degradation by the proteasome. Ubiquitinated by the SCF(FBXW7) complex when phosphorylated at Thr-58 and Ser-62, leading to its degradation by the proteasome. In the nucleoplasm, ubiquitination is counteracted by USP28, which interacts with isoform 1 of FBXW7 (FBW7alpha), leading to its deubiquitination and preventing degradation. In the nucleolus, however, ubiquitination is not counteracted by USP28, due to the lack of interaction between isoform 4 of FBXW7 (FBW7gamma) and USP28, explaining the selective MYC degradation in the nucleolus. Also polyubiquitinated by the DCX(TRUSS) complex.
Cellular localization	Nucleus > nucleoplasm. Nucleus > nucleolus.
Form	c-Myc is also expressed in the cytoplasm.
Images	



Flow Cytometry (Intracellular) - Anti-c-Myc antibody [Y69] - ChIP Grade - BSA and Azide free (ab168727)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32072).

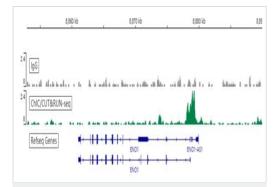
Flow cytometry overlay histogram showing wild-type HEK293 (green line) and MYC knockout HEK293 stained with **ab32072** (red line). The cells were fixed with 80% methanol (5 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (**ab32072**) (1x 10^6 in 100μ l at $0.2~\mu$ g/ml (1/11500)) for 30min at 22° C.

The secondary antibody Goat Anti-Rabbit lgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 for 30min at 22°C

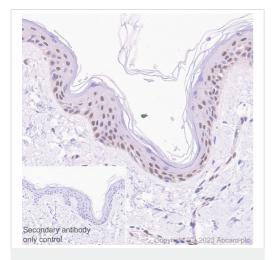
Isotype control antibody Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control was used at the same concentration and conditions as the primary antibody (wild-type HEK293 - black line, MYC knockout HEK293 - grey line). Unlabelled sample was also used as a control (this line is not shown for the purpose of simplicity).

Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.

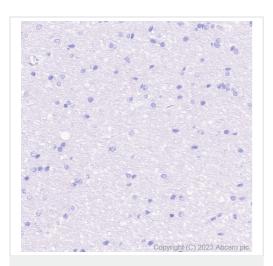
This antibody gave a positive signal in HEK293 Fixed with 4% formaldehyde (10 min) / permeabilised with 0.1% PBS-Triton X-100 for 15 min under the same conditions.



ChIC/CUT&RUN sequencing - Anti-c-Myc antibody
[Y69] - ChIP Grade - BSA and Azide free (ab168727)



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-c-Myc antibody [Y69] - ChIP Grade - BSA and Azide free (ab168727)



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-c-Myc antibody [Y69] - ChIP Grade - BSA and Azide free (ab168727)

ChIC/CUT&RUN was performed using a pAG-MNAse at a final concentration of 700 ng/mL, 2.5×10^5 HeLa (Human cervix adenocarcinoma epithelial cell line) cells and $5 \mu g$ of <u>ab32072</u> [Y69]. The resulting DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 10 million reads. The negative lgG control <u>ab172730</u> is also shown.

Additional screenshots of mapped reads can be downloaded <u>here</u>.

The University of Geneva owns patents relevant to ChlC (Chromatin Immuno-Cleavage) methods.

This data was developed using the same antibody clone in a different buffer formulation (ab32072).

This data was developed using the same antibody clone in a different buffer formulation (ab32072).

Immunohistochemical analysis of Paraffin-embedded sections human skin tissue labelling c-Myc with ab32072 at 1/500 dilution, followed by a ready to use secondary Goat Anti-Rabbit lgG H&L (HRP). Counter stained with Haematoxylin. Secondary antibody only control: Used PBS instead of primary antibody

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins

Positive staining on human skin. The section was incubated with ab32072 for 30 mins at room temperature.

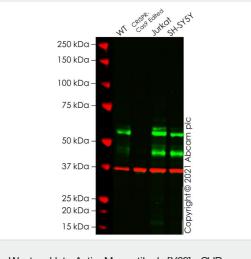
The immunostaining was performed on a Leica Biosystems BOND® RX instrument.

This data was developed using the same antibody clone in a different buffer formulation (ab32072).

Immunohistochemical analysis of Paraffin-embedded sections human cerebrum tissue labelling c-Myc with **ab32072** at 1/500 dilution, followed by a ready to use secondary Goat Anti-Rabbit IgG H&L (HRP). Counter stained with Haematoxylin. Secondary antibody only control: Used PBS instead of primary antibody

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins

Negative control: no staining on human cerebrum. The section was incubated with <u>ab32072</u> for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument.



Western blot - Anti-c-Myc antibody [Y69] - ChIP Grade - BSA and Azide free (ab168727)

All lanes : Anti-c-Myc antibody [Y69] - ChIP Grade (<u>ab32072</u>) at 1/1000 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

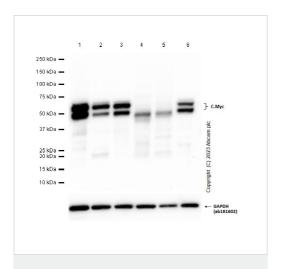
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 48 kDa

Observed band size: 45, 57 kDa

False colour image of Western blot: Anti-c-Myc antibody [Y69] staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab32072 was shown to bind specifically to c-Myc. A band was observed at 45/57 kDa in wildtype 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 45/57 kDa is likely to represent a truncated form of c-Myc. 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 5 % 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-Rabbit lqG H&L (IRDve® 800CW) preabsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (ab216776) at 1/20000 dilution.



Western blot - Anti-c-Myc antibody [Y69] - ChIP Grade - BSA and Azide free (ab168727)

All lanes : Anti-c-Myc antibody [Y69] - ChIP Grade (<u>ab32072</u>) at 1/1000 dilution

Lane 1 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate

Lane 2 : MDA-MB-231 (Human breast adenocarcinoma epithelial cell) whole cell lysate

Lane 3 : DLD-1 (human colorectal adenocarcinoma epithelial cell) whole cell lysate

Lane 4 : HepG2 (Human hepatocellular carcinoma epithelial cell) whole cell lysate

Lane 5: A549 (Human lung carcinoma epithelial cell) whole cell lysate

Lane 6 : HCT 116 (Human colorectal carcinoma epithelial cell) whole cell lysate

Lysates/proteins at 20 µg per lane.

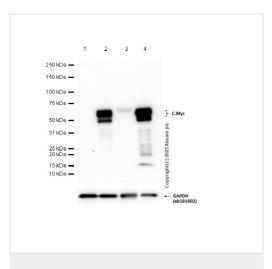
Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 48 kDa **Observed band size:** 45,57 kDa

Exposure time: 20 seconds

Blocking buffer: 5% NFDM/TBST.



Western blot - Anti-c-Myc antibody [Y69] - ChIP Grade - BSA and Azide free (ab168727) **All lanes :** Anti-c-Myc antibody [Y69] - ChIP Grade (**ab32072**) at 1/1000 dilution

Lane 1: Mouse brain tissue lysate

Lane 2: Mouse brain cancer tissue lysate

Lane 3: Mouse skin tissue lysate

Lane 4: Mouse skin cancer tissue lysate

Lysates/proteins at 20 µg per lane.

Secondary

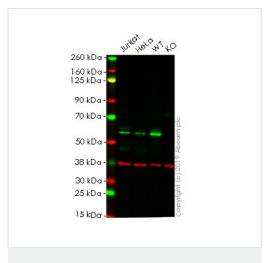
All lanes: Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/20000

dilution

Predicted band size: 48 kDa **Observed band size:** 45,57 kDa

Exposure time: 60 seconds

Blocking buffer: 5% NFDM/TBST.



Western blot - Anti-c-Myc antibody [Y69] - ChIP Grade - BSA and Azide free (ab168727)

All lanes : Anti-c-Myc antibody [Y69] - ChIP Grade (<u>ab32072</u>) at 1/1000 dilution

Lane 1 : Jurkat cell lysate

Lane 2 : HeLa cell lysate

Lane 3: Wild-type HEK-293T cell lysate

Lane 4: MYC knockout HEK-293T cell lysate

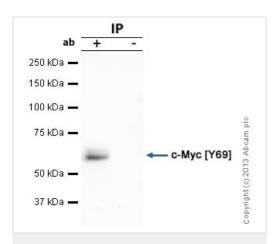
Lysates/proteins at 20 µg per lane.

Predicted band size: 48 kDa **Observed band size:** 57 kDa

This data was developed using the same antibody clone in a different buffer formulation (<u>ab32072</u>).

Lanes 1 - 4: Merged signal (red and green). Green - <u>ab32072</u> observed at 57 kDa. Red - loading control, <u>ab8245</u> observed at 37 kDa.

ab32072 was shown to react with MYC in wild-type HEK-293T cells. Loss of signal was observed when knockout cell line ab256500 (knockout cell lysate ab263850) was used. Wild-type and MYC knockout samples were subjected to SDS-PAGE. ab32072 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated overnight at 4°C at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Immunoprecipitation - Anti-c-Myc antibody [Y69] - ChIP Grade - BSA and Azide free (ab168727)

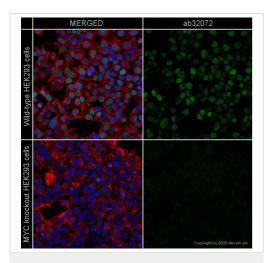
c-Myc was immunoprecipitated using 0.5mg Jurkat (human T cell leukemia cell line from peripheral blood) whole cell extract, 5µg of unpurified rabbit monoclonal to c-Myc [Y69] and 50µl of protein G magnetic beads (+). No antibody was added to the control (-).

The antibody was incubated under agitation with Protein G beads for 10min, Jurkat whole cell extract lysate diluted in RIPA buffer was added to each sample and incubated for a further 10min under agitation.

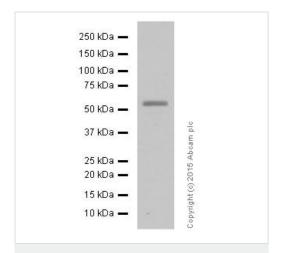
Proteins were eluted by addition of 40µl SDS loading buffer and incubated for 10min at 70°C; 10µl of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with unpurified <u>ab32072</u>.

Secondary: Goat polyclonal to mouse $\lg G$ light chain specific (HRP) at 1/20,000 dilution.

Band: 57kDa; c-Myc [Y69]



Immunocytochemistry/ Immunofluorescence - Antic-Myc antibody [Y69] - ChIP Grade - BSA and Azide free (ab168727)



Western blot - Anti-c-Myc antibody [Y69] - ChIP Grade - BSA and Azide free (ab168727)

This data was developed using the same antibody clone in a different buffer formulation (ab32072). ab32072 staining MYC in wild-type HEK293 cells (top panel) and MYC knockout HEK293 cells (ab256500) (bottom panel). The cells were fixed with 4% paraformaldehyde (10 min) then permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab32072 at 5µg/ml concentration and ab7291 (Mouse monoclonal to alpha Tubulin) at 1/1000 dilution overnight at 4°C followed by a further incubation at room temperature for 1h with a goat secondary antibody to rabbit IgG (Alexa Fluor® 488) (ab150081) at 2 µg/ml (shown in green) and a goat secondary antibody to mouse IgG (Alexa Fluor® 594) (ab150120) at 2 µg/ml (shown in red). Nuclear DNA was labelled in blue with DAPI. Image was taken with a confocal microscope (Leica-Microsystems TCS SP8).

Anti-c-Myc antibody [Y69] - ChIP Grade - BSA and Azide free (ab168727) + Raji (human Burkitt's lymphoma) whole cell lysate at $15~\mu g$

Secondary

Goat Anti-Rabbit IgG H&L (HRP) (ab97051)

Predicted band size: 48 kDa **Observed band size:** 57 kDa

Exposure time: 10 seconds

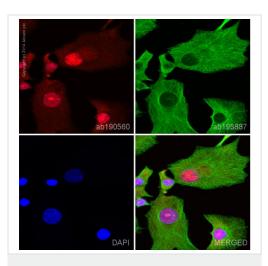
Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM/TBST.



This data was developed using <u>ab32072</u>, the same antibody clone in a different buffer formulation. Different batches of <u>ab32072</u> were tested on Raji (Human Burkitt's lymphoma B lymphocyte) whole cell lysate at 0.2 μ g/ml. 15 μ g of lysate was loaded in each lane. Bands observed at 57 kDa.

Western blot - Anti-c-Myc antibody [Y69] - ChIP Grade - BSA and Azide free (ab168727)



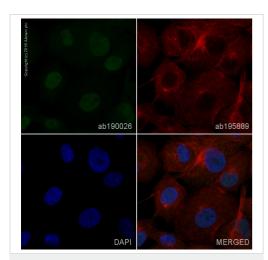
Immunocytochemistry/ Immunofluorescence - Antic-Myc antibody [Y69] - ChIP Grade - BSA and Azide free (ab168727)

Clone Y69 (ab168727) has been successfully conjugated by Abcam. This image was generated using Anti-c-Myc antibody [Y69] (Alexa Fluor® 647). Please refer to ab190560 for protocol details.

ab190560 staining c-Myc in panc1 cells. The cells were fixed with 100% methanol (5 min), permeabilized in 0.1% Triton X-100 for 5 minutes and then blocked in 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with **ab190560** at a working dilution of 1 in 100 (shown in red) and **ab195887**, Mouse monoclonal [DM1A] to alpha Tubulin (Alexa Fluor[®] 488, shown in green) at 2μg/ml overnight at +4°C. Nuclear DNA was labelled in blue with DAPI.

This product also gave a positive signal in 4% formaldehyde (10 min) fixed panc1 cells under the same testing conditions.

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

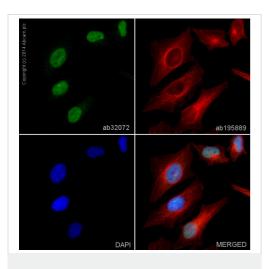


Immunocytochemistry/ Immunofluorescence - Antic-Myc antibody [Y69] - ChIP Grade - BSA and Azide free (ab168727)

Clone Y69 (ab168727) has been successfully conjugated by Abcam. This image was generated using Anti-c-Myc antibody [Y69] (Alexa Fluor® 488). Please refer to ab190026 for protocol details.

ab190026 staining c-myc in Panc-1 cells. The cells were fixed with 4% formaldehyde (10 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with **ab190026** at 1/100 dilution (shown in green) and **ab195889**, Mouse monoclonal to alpha Tubulin (Alexa Fluor[®] 594), at 1/250 dilution (shown in red). Nuclear DNA was labelled with DAPI (shown in blue).

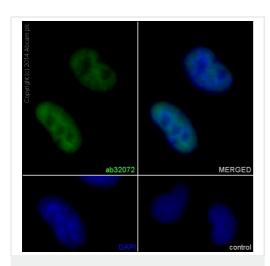
Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Immunocytochemistry/ Immunofluorescence - Antic-Myc antibody [Y69] - ChIP Grade - BSA and Azide free (ab168727)

<u>ab32072</u> staining c-Myc in HeLa (human epithelial cell line from cervix adenocarcinoma) cells. The cells were fixed with 4% formaldehyde (10min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated overnight at +4°C with <u>ab32072</u> at 10μ g/ml dilution (shown in green) and <u>ab195889</u>, mouse monoclonal to alpha Tubulin (Alexa Fluor® 594), at 2μ g/ml (shown in red). Nuclear DNA was labelled with DAPI (shown in blue).

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



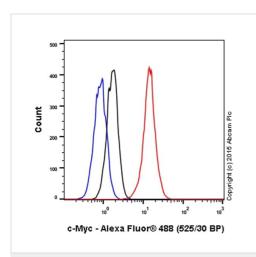
Immunocytochemistry/ Immunofluorescence - Antic-Myc antibody [Y69] - ChIP Grade - BSA and Azide free (ab168727)

Immunocytochemistry/immunofluorescence analysis of HeLa (human epithelial cell line from cervix adenocarcinoma) cells labelling c-Myc with purified ab32072 at 1/100. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody (1/500) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain.

Control: primary antibody (1/100) and secondary antibody, <u>Goat</u>

<u>Anti-Mouse IgG H&L (Alexa Fluor® 594) preadsorbed</u>
(ab150120) (1/500).

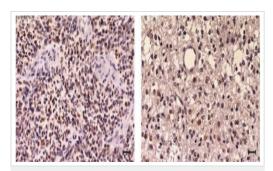
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32072).



Flow Cytometry (Intracellular) - Anti-c-Myc antibody [Y69] - ChIP Grade - BSA and Azide free (ab168727)

Overlay histogram showing HeLa (human epithelial cell line from cervix adenocarcinoma) cells stained with <u>ab32072</u> (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 nonspecific protein-protein interactions followed by the antibody (<u>ab32072</u>, 1/76 dilution) for 30 min at 22°C. The secondary antibody used was Goat Anti-Rabbit IgG H&L (Alexa Fluor[®] 488) preadsorbed (<u>ab150081</u>) secondary antibody at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG [EPR25A] (monoclonal) (<u>ab172730</u>, 1µg/1x10⁶ cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control.

Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 nm bandpass filter.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-c-Myc antibody [Y69] - ChIP Grade - BSA and Azide free (ab168727)

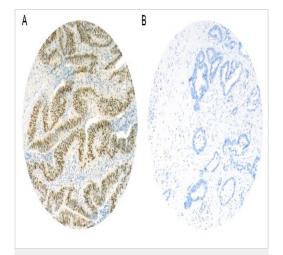
Image from Simeone P et al. PLoS One. 2014;;9(7):e103030.; doi: 10.1371/journal.pone.0103030.

Expression of c-Myc, as determined

by immunohistochemical staining of glioblastoma sample (left) and low-grade glioma tumor (right) with <u>ab32072</u>. Representative samples are shown. Scale bars = $20 \mu m$. Nuclei were counterstained with hematoxylin (in blue).

For the full image see PMID 25050814.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32072).



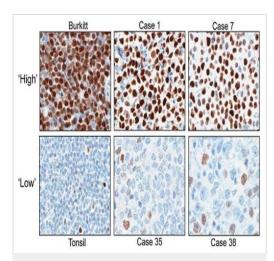
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-c-Myc antibody [Y69] - ChIP Grade - BSA and Azide free (ab168727)

Image from Toon CW et al. PLoS One. 2014;9(2):e87456. Fig 1.; doi: 10.1371/journal.pone.0087456.

Human colorectal carcinoma (CRC) tissues stained for c-Myc using **ab32072** at 1/100 dilution in immunohistochemical analysis.

Panel A: c-Myc positive IHC staining; Panel B: c-Myc negative IHC staining.

For the full image see PMID 24503701.

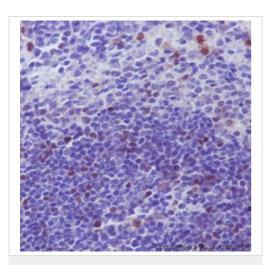


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-c-Myc antibody [Y69] - ChIP Grade - BSA and Azide free (ab168727)

Image from Kluk MJ et al. PLoS One. 2012;7(4):e33813. Fig 1.; doi: 10.1371/journal.pone.0033813.

Photomicrographs of select tumors and reactive tissue stained for c-Myc (positive staining = brown nuclei). Positive control (Burkitt lymphoma with a confirmed *c-Myc* translocation) revealed uniform, intense staining in >90% of tumor cells (Burkitt). In contrast, reactive lymphoid tissue revealed variable staining in only 10% of normal lymphocyte nuclei (Tonsil). Representative images from Diffuse large B cell lymphoma (DLBCL) cases and associated percent c-Myc+ tumor nuclei: Case 1, 90% MYC+; Case 7, 70% MYC+; and Cases 35 and 38, 30% c-Myc+. c-Myc staining was exclusively nuclear in all cases under the described staining conditions.

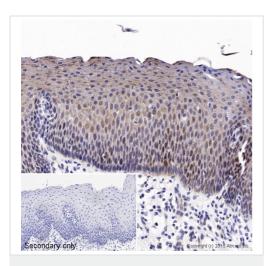
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32072).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-c-Myc antibody [Y69] - ChIP Grade - BSA and Azide free (ab168727)

Immunohistochemical analysis of paraffin-embedded Mouse spleen tissue labeling c-Myc with ab32072 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) secondary antibody at 1/500 dilution. Nuclear staining on mouse spleen. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is **Goat Anti-Rabbit IgG H&L (HRP) (ab97051) secondary antibody** at 1/500 dilution.

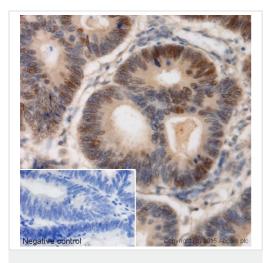


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-c-Myc antibody [Y69] - ChIP Grade - BSA and Azide free (ab168727)

IHC image of <u>ab32072</u> staining c-Myc in human esophagus formalin fixed paraffin embedded tissue sections*, performed on a Leica Bond. 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 <u>ab32072</u>, 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. No primary antibody was used in the Secondary only control (shown on the inset).

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.

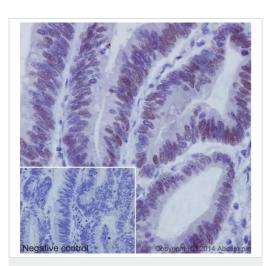
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32072).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-c-Myc antibody [Y69] - ChIP Grade - BSA and Azide free (ab168727)

IHC image of <u>ab32072</u> staining c-Myc in human adenocarcinoma formalin fixed paraffin embedded tissue sections, performed on a Leica Bond. 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 <u>ab32072</u>, 5µg/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. No primary antibody was used in the negative control (shown on the inset).

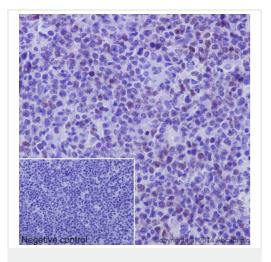
For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-c-Myc antibody [Y69] - ChIP Grade - BSA and Azide free (ab168727)

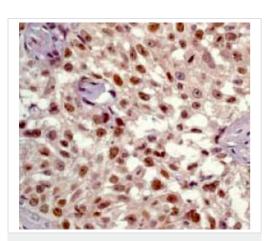
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human adenocarcinoma of the colon tissue labelling c-Myc with purified ab32072 at 1/500. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. Goat antibody was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32072).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-c-Myc antibody [Y69] - ChIP Grade - BSA and Azide free (ab168727)

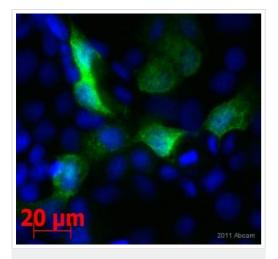
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human diffuse large B cell lymphoma tissue labelling c-Myc with purified ab32072 at 1/500. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. Goat antibody was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-c-Myc antibody [Y69] - ChIP Grade - BSA and Azide free (ab168727)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human skin carcinoma tissue labelling c-Myc with unpurified <u>ab32072</u> at 1/50.

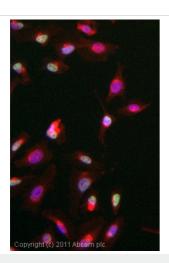
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32072).



Immunocytochemistry/ Immunofluorescence - Antic-Myc antibody [Y69] - ChIP Grade - BSA and Azide free (ab168727)

This image is courtesy of an Abreview submitted by Dr Vladimir Mlenkovic.

Unpurified <u>ab32072</u> staining c-Myc in HEK293 cells transfected with CACNB4-c-Myc by immunocytochemistry/ immunofluorescence. Cells were fixed in paraformal dehyde, permeabilized with 0.5% Triton X-100 then blocked using 5% serum for 20 minutes at 25°C. Samples were then incubated with <u>ab32072</u> at a 1/250 dilution for 16 hours at 4°C. The secondary used was an Alexa Fluor[®] 488 conjugated goat anti-rabbit polyclonal, used at a 1/500 dilution.

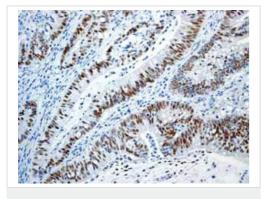


Immunocytochemistry/ Immunofluorescence - Antic-Myc antibody [Y69] - ChIP Grade - BSA and Azide free (ab168727)

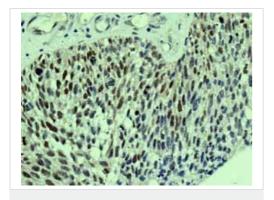
ICC/IF image of unpurified <u>ab32072</u> stained HeLa (human epithelial cell line from cervix adenocarcinoma) cells. The cells were 4% PFA fixed (10 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (<u>ab32072</u>, 1μg/ml) overnight at +4°C. The secondary antibody (green) was <u>Goat Anti-Rabbit IgG H&L (DyLight® 488)</u> <u>preadsorbed (ab96899)</u> used at a 1/250 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32072).

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human adenocarinoma of colon tissue labelling c-Myc with unpurified **ab32072**.



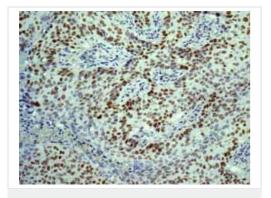
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-c-Myc antibody [Y69] - ChIP Grade - BSA and Azide free (ab168727)



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-c-Myc antibody [Y69] - ChIP Grade - BSA and Azide free (ab168727)

Immunohistochemistry (Formalin/PFA-fixed parffin-embedded sections) analysis of human urinary bladder transitional carcinoma tissue labelling c-Myc with unpurified <u>ab32072</u>.

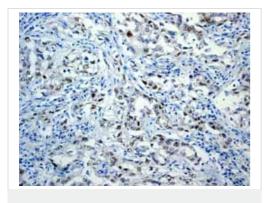
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32072).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-c-Myc antibody [Y69] - ChIP Grade - BSA and Azide free (ab168727)

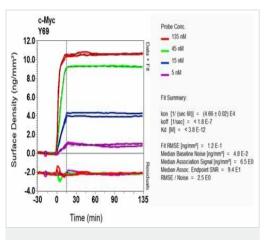
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human lung adenocarinoma tissue labelling c-Myc with unpurified ab32072.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32072).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-c-Myc antibody [Y69] - ChIP Grade - BSA and Azide free (ab168727)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human gastric adenocarcinoma tissue labelling c-Myc with unpurified <u>ab32072</u>.



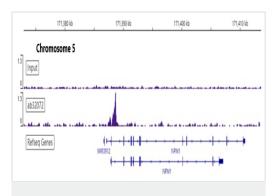
OI-RD Scanning - Anti-c-Myc antibody [Y69] - ChIP Grade - BSA and Azide free (ab168727)

Equilibrium disassociation constant (K_D)

Learn more about K_D

Click here to learn more about KD

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32072).

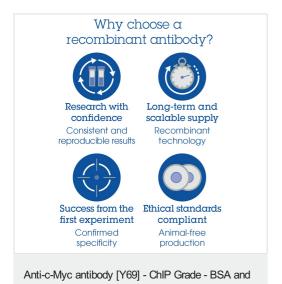


ChIP-sequencing - Anti-c-Myc antibody [Y69] - ChIP Grade - BSA and Azide free (ab168727)

Chromatin was prepared from HeLa cells. Cells were fixed with 1% formaldehyde for 10 minutes. ChIP was performed with 10^7 HeLa cells and $8\mu g$ of <u>ab32072</u> [Y69]. ChIP DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 30 million reads.

Additional screenshots of mapped reads can be downloaded **here**.

This data was developed using the same antibody clone in a different buffer formulation (<u>ab32072</u>).



Azide free (ab168727)

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit https://www.abcam.com/abpromise or contact our technical team.

Terms and conditions

• Guarantee only valid for products bought direct from Abcam or one of our authorized distributors