

Anti-c-Myc antibody [Y69] - ChIP Grade

Anti-c-Myc antibody [Y69] - ChIP Grade (ab32072) is a rabbit monoclonal antibody detecting c-Myc in **Western Blot, Flow Cytometry (Intra), IP, IHC-P, ICC/IF, ChIP-seq, ChIC/CUT&RUN-seq**. Suitable for **Human, Mouse, Rat** samples.

- Enhanced IHC validation data** - Download the data
- detailed IHC expression profiling in normal and clinically relevant cancer FFPE TMAs- ovarian, stomach and prostate
 - protocols for BOND RX™ and Ventana DISCOVERY ULTRA
- KO validated** for confirmed specificity
- Over 1320 publications**
- Trusted since 2006**
- Biophysical QC** for unrivalled batch-batch consistency

Lab Essentials

Recombinant

RabMAb

Advanced Validation

KO Validated

20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	Y69
Purification technique	Affinity purification Protein A
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. Please see documentation for further information on positive controls (Chinese version).
Dissociation constant	3.8 x 10 ⁻¹² M

Concentration	0.495 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →
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Reactivity data

IHC-P

Tested	
Species	Human
Dilution info	5 µg/mL
Notes	ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody. Perform heat-mediated antigen retrieval before commencing with IHC staining protocol.

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

IP

Tested	
Species	Human
Dilution info	5 µg/mL
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

WB

Tested

Species	Mouse
Dilution info	1/1000
Notes	-

Species	Rat
Dilution info	1/1000
Notes	-

Species	Human
Dilution info	1/1000
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	5-10 µg/mL
Notes	1/100.

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

ChIC/CUT&RUN-seq

Tested

Species	Human
Dilution info	5 µg
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

ChIP-seq

Tested

Species	Human
Dilution info	8 µg for 10 ⁷ Cells
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/50
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Target data

[See full target information MYC](#) 

Function	Transcription factor that binds DNA in a non-specific manner, yet also specifically recognizes the core sequence 5'-CAC[GA]TG-3' (PubMed:24940000, PubMed:25956029). Activates the transcription of growth-related genes (PubMed:24940000, PubMed:25956029). Binds to the VEGFA promoter, promoting VEGFA production and subsequent sprouting angiogenesis (PubMed:24940000, PubMed:25956029). Regulator of somatic reprogramming, controls self-renewal of embryonic stem cells (By similarity). Functions with TAF6L to activate target gene expression through RNA polymerase II pause release (By similarity). Positively regulates transcription of HNRNPA1, HNRNPA2 and PTBP1 which in turn regulate splicing of pyruvate kinase PKM by binding repressively to sequences flanking PKM exon 9, inhibiting exon 9 inclusion and resulting in exon 10 inclusion and production of the PKM M2 isoform (PubMed:20010808).
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Storage

Shipped at conditions	Conditional Ambient
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

Notes

Anti-c-Myc antibody [Y69] - ChIP Grade (ab32072) was developed by Abcam using patented rabbit monoclonal antibody technology and is validated for use in ChIC/CUT&RUN-seq, ChIP,-seq Flow Cyt (Intra), ICC/IF, IHC-P and IP.

Anti-c-Myc antibody [Y69] - ChIP Grade (ab32072) was first used in a scientific publication in 1985 and has been cited over 1320 times in peer reviewed journals. It's performance in Western blot and IHC in human and mouse samples is trusted by the scientific community.

Abcam's high quality manufacturing and validation processes ensure Anti-c-Myc antibody [Y69] - ChIP Grade (ab32072) has high sensitivity and specificity alongside high lot-to-lot consistency and reproducibility.

The specificity of Anti-c-Myc antibody [Y69] - ChIP Grade (ab32072) has been confirmed by Western Blot testing in c-Myc knockout HEK-293T cells (ab256500).

Anti-c-Myc antibody [Y69] - ChIP Grade (ab32072) has 55 independent reviews from customers.

c-Myc antibodies are used to visualize proteins labelled with this tag in a variety of applications (for example imaging and Flow cytometry). To enable specific detection of your tagged protein, Anti-c-Myc antibody [Y69] - ChIP Grade

(ab32072) has been validated in ab256500 ChIC/CUT&RUN-seq, ChIP,-seq Flow Cyt (Intra), ICC/IF, IHC-P and IP.

Anti-c-Myc antibody [Y69] - ChIP Grade (ab32072) specifically detects c-Myc (UniProt ID: P01106; Molecular weight: 51kDa) and is sold in a convenient trial size to enable initial testing (20 µL) and larger sizes for subsequent scaling up experiments (100 µL and 1 mL).

Conjugation-ready, carrier free format available for antibody clone Y69 - ab168727.

Antibody clone Y69 is also available pre-conjugated to a variety of labels for your convenience - Alexa Fluor® 488, Alexa Fluor® 647, Alexa Fluor® 594, Alexa Fluor® 555, HRP, FITC (ab190026, ab190560, ab201775, ab201780, ab205818, ab223913).

Only antibody in the market to this target validated for both CUT&RUN and ChIP-seq, which are key applications to map protein-DNA interactions on a genome-wide scale using NGS. Highly-specific antibody targeting c-Myc, a crucial transcription factor involved in various cellular processes including cancer biology, cell cycle regulation and apoptosis studies. Widely used in cancer research, this antibody is crucial for investigating c-Myc's role in tumorigenesis, including Burkitt lymphoma, multiple myeloma and glioblastoma.

Patented technology

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

What are the advantages of a recombinant monoclonal antibody?

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 batch production

For more information, read more on recombinant antibodies.

Supplementary info

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

Activity summary

The c-Myc protein also known as Myc or cMyc is a transcription factor with a molecular weight of approximately 49 kDa. It plays a mechanical role by binding to DNA where it regulates the expression of various genes. Researchers often detect c-Myc in the cell nucleus. It expresses in many cell types including embryonic stem cells and cancer cells. Due to its influential role in proliferation and growth scientists have highly studied c-Myc for its functions and implications.

Biological function summary

C-Myc influences cellular processes like cell cycle progression apoptosis and metabolism. It often forms a complex with its partner protein Max to bind target genes and activate transcription. This c-Myc/Max complex operates by manipulating gene expression patterns which leads to changes in cellular behavior. Through its activity c-Myc affects cell growth and division which remain critical for development and homeostasis.

Pathways

Many scientists understand that c-Myc serves major functions in the MYC signaling pathway and the PI3K/AKT signaling pathway. c-Myc modulates the expression of

genes that govern cell cycle checkpoints and cellular metabolism through these pathways. In this context c-Myc interacts with proteins like TCF and cyclins allowing the fine-tuning of cell proliferation and growth signals. These pathway involvements highlight the protein's importance in fundamental cellular mechanisms and its regulation.

Associated diseases and disorders

Researchers have frequently associated c-Myc with cancer and cardiovascular disorders. In cancer c-Myc overexpression can drive tumorigenesis by promoting uncontrolled cell proliferation. This often involves interactions with other oncogenic proteins like Bcl-2. In cardiovascular disorders c-Myc dysregulation can disrupt normal cellular function and contribute to pathophysiological conditions. Studies continue to explore c-Myc's role in these diseases aiming to develop therapeutic strategies targeting its activity.

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

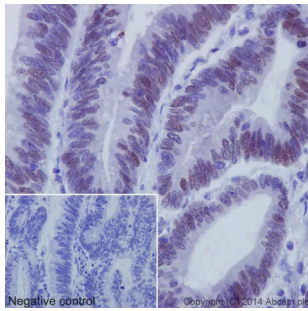
We do not recommend this combination. It is not covered by our product promise.

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](#).

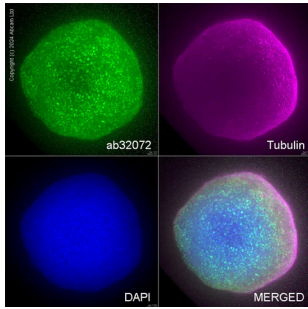
32 product images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-c-Myc antibody [Y69] - ChIP Grade (ab32072)

C-Myc Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of human adenocarcinoma of the colon tissue using rabbit Anti-c-Myc antibody

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human adenocarcinoma of the colon tissue labelling c-Myc with purified ab32072 at 1/500 dilution. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) secondary antibody was used as the secondary antibody (1/500 dilution). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.



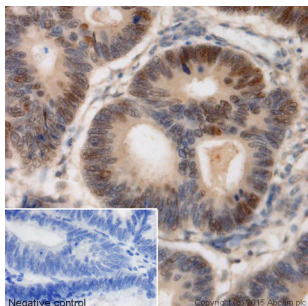
Immunocytochemistry/ Immunofluorescence - Anti-c-Myc antibody [Y69] - ChIP Grade (ab32072)

C-Myc Immunocytochemistry/ Immunofluorescence staining of HCT 116 cell spheroid using rabbit Anti-c-Myc antibody

ab32072 staining of Myc proto-oncogene protein in a HCT 116 cell spheroid. The cells were fixed with 4% paraformaldehyde (10 min), permeabilised with 2% Triton X-100 for 1h and then blocked with 1% BSA/10% normal goat serum/0.3M glycine 0.1% PBS-Tween overnight at room temperature. The spheroids were then incubated overnight at room temperature with ab32072 at 2 µg/ml and [ab7291](#), Mouse monoclonal [DM1A] to alpha Tubulin at 2 µg/ml. DAPI was used as nuclear counterstain (shown in blue). As secondary antibodies [ab150081](#) Goat anti-Rabbit (Alexa Fluor® 488) (shown in green) and [ab150120](#) Goat anti-Mouse (Alexa Fluor® 594) (shown in magenta) were used, incubated overnight at room temperature. All permeabilization, blocking and antibody incubation steps were performed using a rotary shaker.

Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown.

Similar results were observed using 100% Methanol (5min).

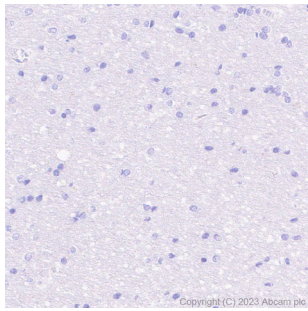


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-c-Myc antibody [Y69] - ChIP Grade (ab32072)

C-Myc Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of human adenocarcinoma tissue using rabbit Anti-c-Myc antibody

IHC image of ab32072 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 ab32072, 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).

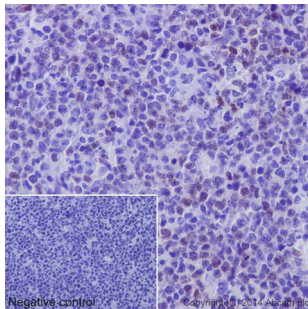
For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-c-Myc antibody [Y69] - ChIP Grade (ab32072)

C-Myc Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Human cerebrum tissue using rabbit Anti-c-Myc antibody

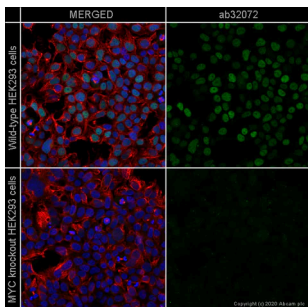
Immunohistochemical analysis of Paraffin-embedded sections human cerebrum tissue labelling c-Myc with ab32072 at 1/500 dilution, followed by a ready to use secondary Goat Anti-Rabbit IgG H&L (HRP). Counter stained with Haematoxylin. Secondary antibody only control: Used PBS instead of primary antibody 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 ab32072 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-c-Myc antibody [Y69] - ChIP Grade (ab32072)

C-Myc Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-c-Myc antibody

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 Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) secondary antibody was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.

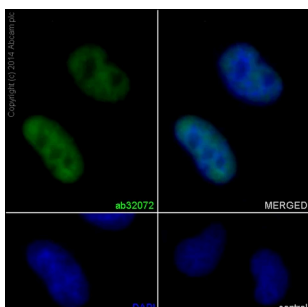


Immunocytochemistry/ Immunofluorescence - Anti-c-Myc antibody [Y69] - ChIP Grade (ab32072)

C-Myc Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-c-Myc antibody

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).

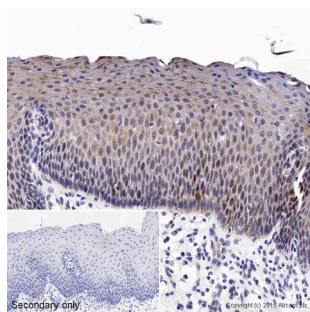


Immunocytochemistry/ Immunofluorescence - Anti-c-Myc antibody [Y69] - ChIP Grade (ab32072)

C-Myc Immunocytochemistry/ Immunofluorescence staining of HeLa cells using rabbit Anti-c-Myc antibody

Immunocytochemistry/immunofluorescence analysis of HeLa (human epithelial cell line from cervix adenocarcinoma) cells labelling c-Myc with purified ab32072 at 1/100 dilution. 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 dilution) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain.

Control: primary antibody (1/100 dilution) and secondary antibody Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) preadsorbed ([ab150120](#)) (1/500 dilution).

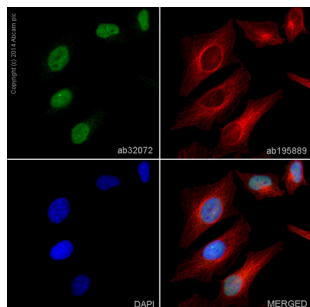


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-c-Myc antibody [Y69] - ChIP Grade (ab32072)

C-Myc Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of human esophagus using rabbit Anti-c-Myc antibody

IHC image of ab32072 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 ab32072, 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.



Immunocytochemistry/ Immunofluorescence - Anti-c-Myc antibody [Y69] - ChIP Grade (ab32072)

C-Myc Immunocytochemistry/ Immunofluorescence staining of HeLa cells using rabbit Anti-c-Myc antibody

ab32072 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 ab32072 at 10µg/ml dilution (shown in green) and [ab195889](#) mouse monoclonal to alpha Tubulin (Alexa Fluor® 594) at 2µg/ml (shown in red). Nuclear DNA was labelled with DAPI (shown in blue).

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

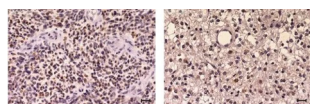


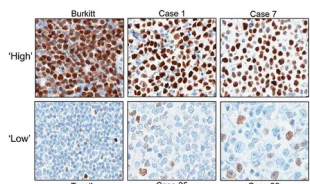
Image from Simeone P et al. PLoS One. 2014;;9(7):e103030.; doi: 10.1371/journal.pone.0103030.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-c-Myc antibody [Y69] - ChIP Grade (ab32072)

C-Myc Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-c-Myc antibody

Expression of c-Myc, as determined by immunohistochemical staining of glioblastoma sample (left) and low-grade glioma tumor (right) with ab32072. Representative samples are shown. Scale bars =20 µm. Nuclei were counterstained with hematoxylin (in blue).

For the full image see PMID 25050814.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-c-Myc antibody [Y69] - ChIP Grade (ab32072)

C-Myc Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-c-Myc antibody

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 is the 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.

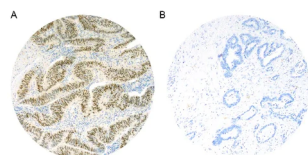


Image from Toon CW et al.
 PLoS One.
 2014;9(2):e87456. Fig 1.;
 doi:
 10.1371/journal.pone.0087456.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-c-Myc antibody [Y69] - ChIP Grade (ab32072)

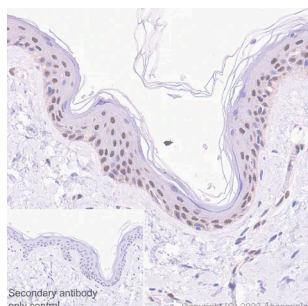
C-Myc Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Human colorectal carcinoma tissue using rabbit Anti-c-Myc antibody

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.

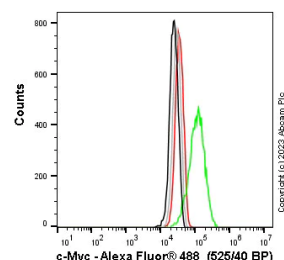
For the full image see PMID 24503701.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-c-Myc antibody [Y69] - ChIP Grade (ab32072)

C-Myc Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Human skin tissue using rabbit Anti-c-Myc antibody

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 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 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.

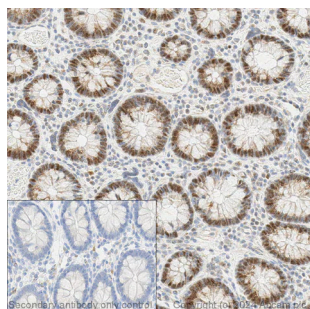


Flow Cytometry (Intracellular) - Anti-c-Myc antibody [Y69] - ChIP Grade (ab32072)

C-Myc Flow Cytometry (Intracellular) staining of c-MYC KO cells using rabbit Anti-c-Myc antibody

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 10⁶ in 100µl at 0.2 µg/ml (1/11500 dilution)) for 30min at 22°C. The secondary antibody Goat Anti-Rabbit IgG 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

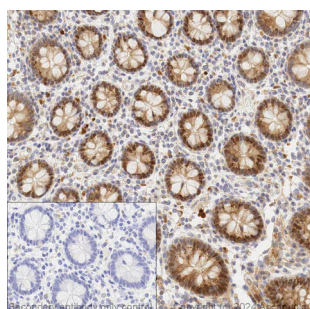
positive signal in HEK293 Fixed with 4% formaldehyde (10 min) / permeabilised with 0.1% PBS-Triton X-100 for 15 min under the same conditions.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-c-Myc antibody [Y69] - ChIP Grade (ab32072)

C-Myc Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of human colon using rabbit Anti-c-Myc antibody

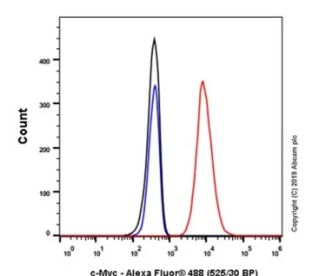
Immunohistochemical analysis of formalin fixed paraffin embedded human colon labelling c-Myc with ab32072 at a concentration of 1 µg/ml. The immunostaining was performed on a Ventana DISCOVERY ULTRA (Roche Tissue Diagnostics) instrument with an OptiView DAB IHC Detection Kit. Heat mediated antigen retrieval was conducted for 32 mins at 100°C with ULTRA cell conditioning solution (CC1, pH 8.5). ab32072 anti-c-Myc antibody [Y69] was incubated at 37°C for 16 mins. Sections were counterstained with Hematoxylin II. Image inset shows absence of staining in secondary antibody only control.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-c-Myc antibody [Y69] - ChIP Grade (ab32072)

C-Myc Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of human colon using rabbit Anti-c-Myc antibody

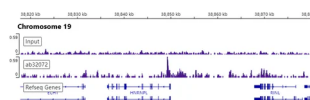
Immunohistochemical analysis of formalin fixed paraffin embedded human colon labelling c-Myc with ab32072 at a concentration of 1 µg/ml. The immunostaining was performed on a Leica Biosystems BOND® RX instrument with a Bond™ Polymer Refine Detection kit. Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution 2) for 20 mins. ab32072 anti-c-Myc antibody [Y69] was incubated for 30 mins at room temperature. Sections were counterstained with Hematoxylin. Image inset shows absence of staining in secondary antibody only control.



Flow Cytometry (Intracellular) - Anti-c-Myc antibody [Y69] - ChIP Grade (ab32072)

C-Myc Flow Cytometry (Intracellular) staining of HeLa (Human cervix adenocarcinoma epithelial cell) cells using rabbit Anti-c-Myc antibody

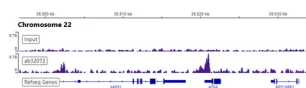
Intracellular Flow Cytometry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) labelling c-Myc with ab32072 at 1/50 (red). Cells were fixed with 4% paraformaldehyde. A Goat anti rabbit IgG (Alexa Fluor® 488 [ab150077](#) (1/2000) was used as the secondary antibody. Black - Isotype control, ([ab172730](#)) rabbit monoclonal IgG. Blue - Unlabelled control, cells without incubation with primary and secondary antibodies.



ChIP-seq - Anti-c-Myc antibody [Y69] - ChIP Grade (ab32072)

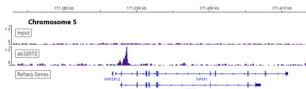
Chromatin was prepared from HeLa cells. Cells were fixed with 1% formaldehyde for 10 minutes. ChIP was performed with 10⁷ NCCIT cells and 8µg of ab32072 [Y69]. ChIP DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 30 million reads.

Additional screenshots of mapped reads can be downloaded [here](#).



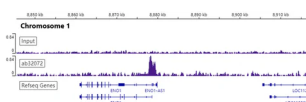
ChIP-sequencing - Anti-c-Myc antibody [Y69] - ChIP Grade (ab32072)

Chromatin was prepared from HeLa cells. Cells were fixed with 1% formaldehyde for 10 minutes. ChIP was performed with 10^7 NCCIT cells and 8µg of ab32072 [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](#).



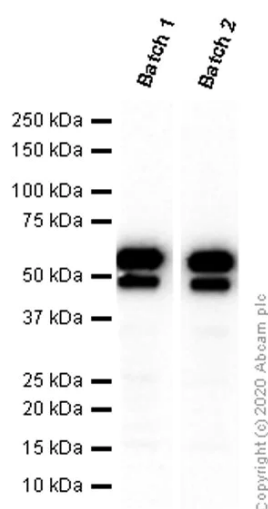
ChIP-sequencing - Anti-c-Myc antibody [Y69] - ChIP Grade (ab32072)

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ChIP-sequencing - Anti-c-Myc antibody [Y69] - ChIP Grade (ab32072)

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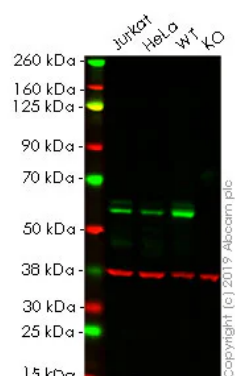
Western blot - Anti-c-Myc antibody [Y69] - ChIP Grade (ab32072)

C-Myc Western blot staining using rabbit Anti-c-Myc antibody

Different batches of ab32072 were tested on Raji (Human Burkitt's lymphoma B lymphocyte) whole cell lysate at 0.2 µg/ml. 15 µg of lysate was loaded in each lane. Bands observed at 57 kDa. All lanes:

Western blot - Anti-c-Myc antibody [Y69] - ChIP Grade (ab32072)

Predicted band size: 48 kDa



Western blot - Anti-c-Myc antibody [Y69] - ChIP Grade (ab32072)

C-Myc Western blot staining using rabbit Anti-c-Myc antibody

Lanes 1 - 4: Merged signal (red and green). Green - ab32072 observed at 57 kDa. Red - loading control, [ab8245](#) 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.

All lanes:

Western blot - Anti-c-Myc antibody [Y69] - ChIP Grade ([ab32072](#)) at 1/1000 dilution

Lane 1:

Jurkat cell lysate at 20 µg

Lane 2:

HeLa cell lysate at 20 µg

Lane 3:

Wild-type HEK-293T cell lysate at 20 µg

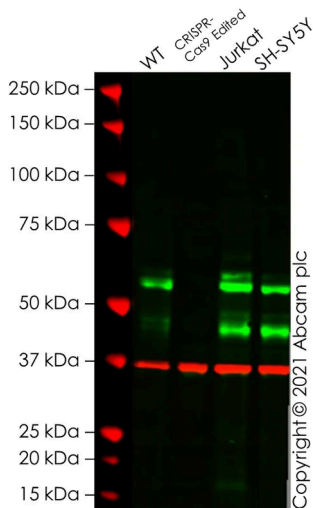
Lane 4:

MYC knockout HEK-293T cell lysate at 20 µg

Secondary

All lanes:

Western blot - Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed ([ab216773](#)) at 1/20000 dilution



Western blot - Anti-c-Myc antibody [Y69] - ChIP Grade ([ab32072](#))

C-Myc Western blot staining using rabbit Anti-c-Myc antibody

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 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 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 IgG H&L (IRDye® 800CW) preabsorbed ([ab216773](#)) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed ([ab216776](#)) at 1/20000 dilution.

All lanes:

Western blot - Anti-c-Myc antibody [Y69] - ChIP Grade ([ab32072](#)) at 1/1000 dilution

Lane 1:

Wild-type HEK-293T cell lysate at 20 µg

Lane 2:

Western blot - Human MYC (c-Myc) knockout HEK-293T cell lysate ([ab263850](#)) at 20 µg

Lane 3:

Jurkat cell lysate at 20 µg

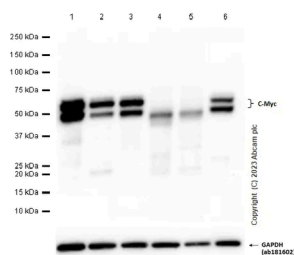
Lane 4:

SH-SY5Y cell lysate at 20 µg

Performed under reducing conditions.

Predicted band size: 48 kDa

Observed band size: 45 kDa, 57 kDa



Western blot - Anti-c-Myc antibody [Y69] - ChIP Grade (ab32072)

C-Myc Western blot staining using rabbit Anti-c-Myc antibody

Blocking buffer: 5% NFDM/TBST.

All lanes:

Western blot - Anti-c-Myc antibody [Y69] - ChIP Grade (ab32072) at 1/1000 dilution

Lane 1:

HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate at 20 µg

Lane 2:

MDA-MB-231 (Human breast adenocarcinoma epithelial cell) whole cell lysate at 20 µg

Lane 3:

DLD-1 (human colorectal adenocarcinoma epithelial cell) whole cell lysate at 20 µg

Lane 4:

HepG2 (Human hepatocellular carcinoma epithelial cell) whole cell lysate at 20 µg

Lane 5:

A549 (Human lung carcinoma epithelial cell) whole cell lysate at 20 µg

Lane 6:

HCT 116 (Human colorectal carcinoma epithelial cell) whole cell lysate at 20 µg

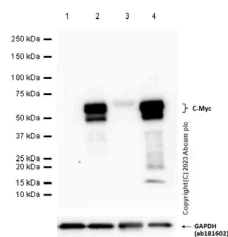
Secondary

All lanes:

Western blot - Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) at 1/20000 dilution

Observed band size: 45 kDa, 57 kDa

Exposure time: 20s



Western blot - Anti-c-Myc antibody [Y69] - ChIP Grade (ab32072)

C-Myc Western blot staining using rabbit Anti-c-Myc antibody

Blocking buffer: 5% NFDM/TBST.

All lanes:

Western blot - Anti-c-Myc antibody [Y69] - ChIP Grade (ab32072) at 1/1000 dilution

Lane 1:

Mouse brain tissue lysate at 20 µg

Lane 2:

Mouse brain cancer tissue lysate at 20 µg

Lane 3:

Mouse skin tissue lysate at 20 µg

Lane 4:

Mouse skin cancer tissue lysate at 20 µg

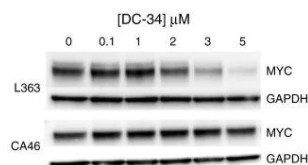
Secondary

All lanes:

Western blot - Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) at 1/20000 dilution

Observed band size: 45 kDa

Exposure time: 60s



This image is taken from Calabrese D. R. et al. Nat Commun. 2018; 9: 4229.

Fig 3e doi:

10.1038/s41467-018-

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Western blot - Anti-c-Myc antibody [Y69] - ChIP Grade (ab32072)

C-Myc Western blot staining using rabbit Anti-c-Myc antibody

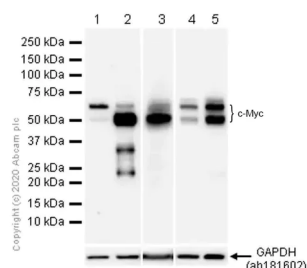
Western blot of L363 MM cell and CA46 cells measuring expression of c-Myc (using ab32072) and GAPDH as the dose of DC-34 increases.

c-Myc protein levels are inhibited as a function of the dose of DC-34 in L363 cells; only the highest dose of DC-34 affected c-Myc in the more resistant CA46 Burkitt's lymphoma cells.

All lanes:

Western blot - Anti-c-Myc antibody [Y69] - ChIP Grade (ab32072)

Predicted band size: 48 kDa



Western blot - Anti-c-Myc antibody [Y69] - ChIP Grade (ab32072)

C-Myc Western blot staining using rabbit Anti-c-Myc antibody

Blocking/Diluting buffer and concentration: 5% NFDM/TBST. Lanes 1 and 2: 80 seconds exposure time. Lanes 3 to 5: 5 seconds exposure time.

All lanes:

Western blot - Anti-c-Myc antibody [Y69] - ChIP Grade (ab32072) at 1/1000 dilution

Lane 1:

Rat pancreas lysates at 20 μg

Lane 2:

AR42J (Rat pancreatic tumor epithelial cell) whole cell lysates at 20 μg

Lane 3:

Rat-1 (Rat embryonic fibroblast) whole cell lysates at 20 μg

Lane 4:

HEK-293 (Human embryonic kidney epithelial cell) whole cell lysates at 20 μg

Lane 5:

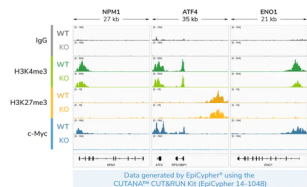
HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates at 20 μg

Secondary

All lanes:

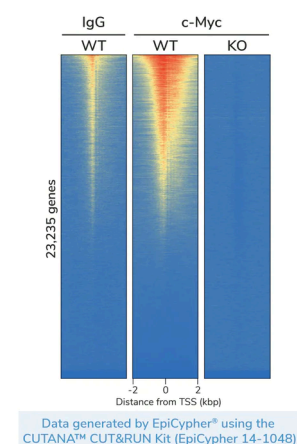
Western blot - Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) at 20000 μg/mL

Observed band size: 45 kDa, 57 kDa



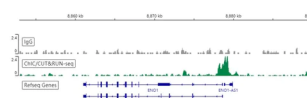
ChIC/CUT&RUN sequencing - Anti-c-Myc antibody [Y69] - ChIP Grade (ab32072)

CUT&RUN profiling with c-Myc antibody reveals the expected genomic enrichment pattern in wild-type (WT) cells, which is substantially reduced in MYC knockout (KO) cells. Representative genome browser tracks show CUT&RUN data generated using the CUTANA™ CUT&RUN Kit (EpiCypher 14-1048) with c-Myc antibody (Abcam ab32072, 0.5 µg). 500,000 HEK293T WT or KO (Abcam [ab256500](#)) cells were used per reaction. IgG, H3K4me3, and H3K27me3 antibodies were included as controls to assess non-specific background, active promoters, and repressed chromatin, respectively. Libraries were prepared using the CUTANA™ CUT&RUN Library Prep Kit (EpiCypher 14-1001). Sequencing was performed with paired-end 50 bp reads, and data were processed on CUTANA™ Cloud (cloud.epicypher.com) by alignment to the hg38 genome. Images were generated using Integrative Genomics Viewer (IGV, Broad Institute).



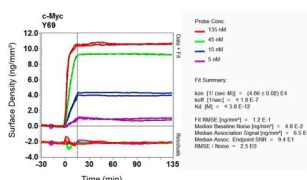
ChIC/CUT&RUN sequencing - Anti-c-Myc antibody [Y69] - ChIP Grade (ab32072)

CUT&RUN profiling with c-Myc antibody demonstrates robust genome-wide enrichment in wild-type (WT) cells, which is markedly diminished in MYC knockout (KO) cells. Heatmaps of genome-wide signal flanking annotated transcription start sites (TSSs, +/- 2 kbp) display CUT&RUN data generated using the CUTANA™ CUT&RUN Kit (EpiCypher 14-1048) with c-Myc antibody (Abcam ab32072, 0.5 µg). 500,000 HEK293T WT or KO (Abcam [ab256500](#)) cells were used per reaction. IgG antibody was included as a negative control to assess non-specific background. Libraries were prepared using the CUTANA™ CUT&RUN Library Prep Kit (EpiCypher 14-1001). Sequencing was performed with paired-end 50 bp reads, and data were processed on CUTANA™ Cloud (cloud.epicypher.com) by alignment to the hg38 genome. Heatmaps were generated using deepTools (Ramirez et al., Nucleic Acids Res. 2014; PMID: 24799436). Row-linked data are ranked by intensity relative to c-Myc WT, with red indicating high localized enrichment and blue denoting background. Validated antibodies show genome-wide enrichment above IgG background consistent with c-Myc binding in WT cells and near complete loss of signal in KO cells.



ChIC/CUT&RUN sequencing - Anti-c-Myc antibody [Y69] - ChIP Grade (ab32072)

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 µg of ab32072 [Y69]. The resulting DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 10 million reads. The negative IgG control [ab172730](#) is also shown. Additional screenshots of mapped reads can be found in the Protocol booklet in the Product Protocol section. The University of Geneva owns patents relevant to ChIC (Chromatin Immuno-Cleavage) methods.



OI-RD Scanning - Anti-c-Myc antibody [Y69] - ChIP Grade (ab32072)

We have systematically measured KD (the equilibrium dissociation constant between the antibody and its antigen), of more than 840 recombinant antibodies to assess not only their individual KD values but also to see the average affinity of antibody. Based on the comparison with published literature values for mouse monoclonal antibodies, Recombinant antibodies appear to be on average 1-2 order of magnitude higher affinity.

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