

## Product datasheet

# Anti-c-Myc antibody [Y69] - ChIP Grade ab32072

KO VALIDATED Recombinant RabMAb

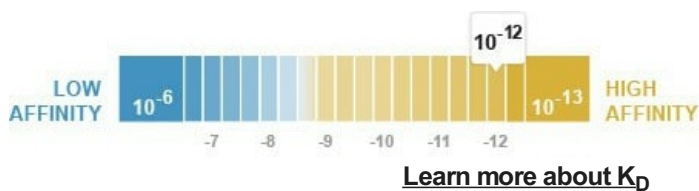
★★★★☆ 55 Abreviews 1156 References 31 Images

### Overview

<b>Product name</b>	Anti-c-Myc antibody [Y69] - ChIP Grade
<b>Description</b>	Rabbit monoclonal [Y69] to c-Myc - ChIP Grade
<b>Host species</b>	Rabbit
<b>Specificity</b>	<p>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.</p> <p><b><u>FURTHER INFORMATION ON POSITIVE CONTROLS (Chinese version)</u></b></p>
<b>Tested applications</b>	<b>Suitable for:</b> Flow Cyt (Intra), WB, ICC/IF, ChIP-sequencing, IHC-P, IP, ChIC/CUT&RUN-seq
<b>Species reactivity</b>	<b>Reacts with:</b> Mouse, Rat, Human
<b>Immunogen</b>	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. (Peptide available as <a href="#">ab166837</a> )
<b>Positive control</b>	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, ChIC/C&R-seq: HeLa cells, HEK293 cells.
<b>General notes</b>	<p>The proto-oncogene MYC plays a role in human oncogenesis. For more information see <a href="#">here</a>.</p> <p><b>This recombinant rabbit monoclonal antibody (Y69) to c-Myc specifically detects endogenous c-Myc. <a href="#">ab32</a>, the Mouse monoclonal antibody (9E10 clone) to the Myc tag however can be used to study Myc-tagged proteins. More information comparing the two antibodies can be found <a href="#">here</a>.</b></p> <p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"><li>- High batch-to-batch consistency and reproducibility</li><li>- Improved sensitivity and specificity</li><li>- Long-term security of supply</li><li>- Animal-free production</li></ul> <p>For more information <a href="#">see here</a>.</p> <p>Our RabMAb<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <a href="#">RabMAb<sup>®</sup> patents</a>.</p>

## 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. Avoid freeze / thaw cycle.
Dissociation constant (K <sub>D</sub> )	K <sub>D</sub> = 3.80 x 10 <sup>-12</sup> M



Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	Y69
Isotype	IgG

## Applications

The **Abpromise guarantee** Our **Abpromise guarantee** covers the use of ab32072 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)		1/76.
WB	★★★★★ (21)	1/1000. Detects a band of approximately 57 kDa (predicted molecular weight: 49 kDa). Can be blocked with <b>Human c-Myc peptide (ab166837)</b> .
ICC/IF	★★★★★ (7)	Use a concentration of 5 - 10 µg/ml. 1/100.
ChIP-sequencing		Use 8µg for 10 <sup>7</sup> cells.
IHC-P	★★★★★ (17)	Use a concentration of 5 µg/ml. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol. <b>ab172730</b> - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
IP	★★★★★ (6)	Use a concentration of 5 µg/ml.
ChIC/CUT&RUN-seq		Use at an assay dependent concentration. 5µg

## 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.

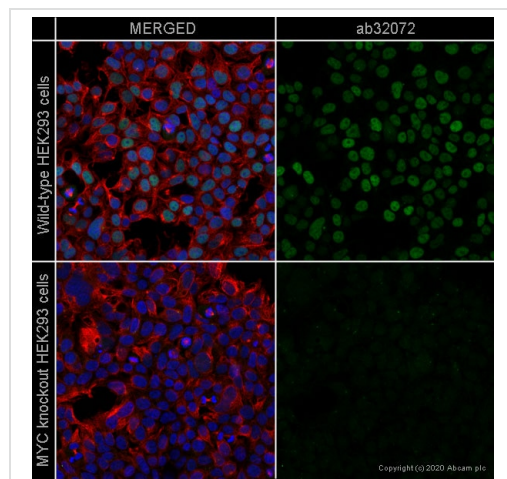
## Cellular localization

Nucleus > nucleoplasm. Nucleus > nucleolus.

## Form

c-Myc is also expressed in the cytoplasm.

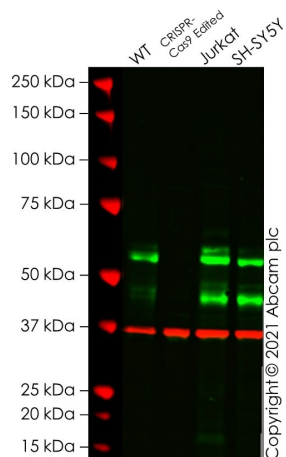
## Images



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



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

**All lanes** : Anti-c-Myc antibody [Y69] - ChIP Grade (ab32072) 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

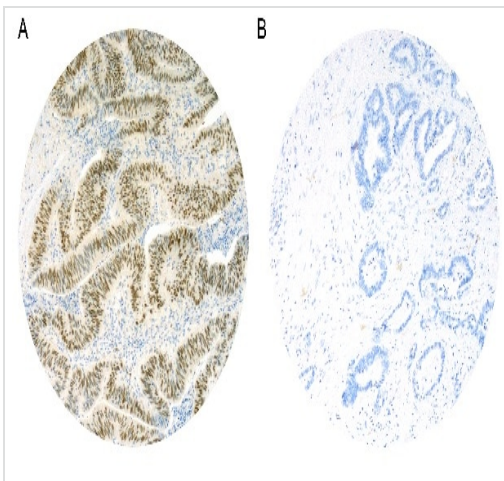
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

**Predicted band size:** 49 kDa

**Observed band size:** 45 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 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<sup>®</sup> 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<sup>®</sup> 800CW) preabsorbed ([ab216773](#)) and Goat anti-Mouse IgG H&L (IRDye<sup>®</sup> 680RD) preabsorbed ([ab216776](#)) at 1/20000 dilution.



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

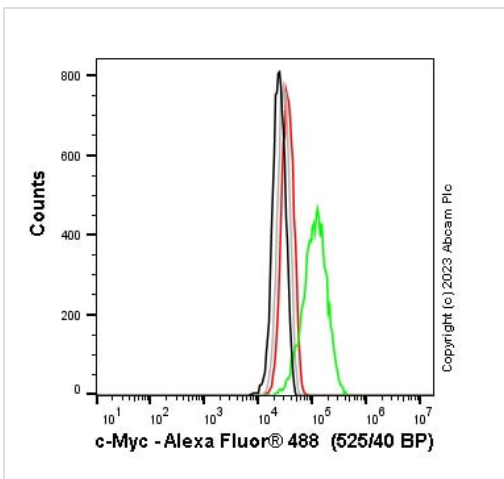
Image from Toon CW et al., PLoS One. 2014;9(2):e87456. Fig 1.; doi: 10.1371/journal.pone.0087456. Reproduced under the Creative Commons license <http://creativecommons.org/licenses/by/4.0/>

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.



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

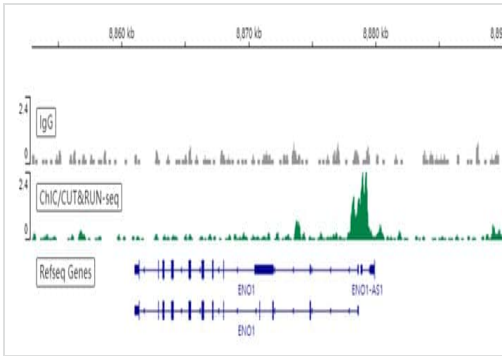
Flow cytometry overlay histogram showing wild-type HEK293 (green line) and MYC knockout HEK293 stained with ab32072 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10% normal goat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

The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 for 30min at  $22^\circ\text{C}$

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.

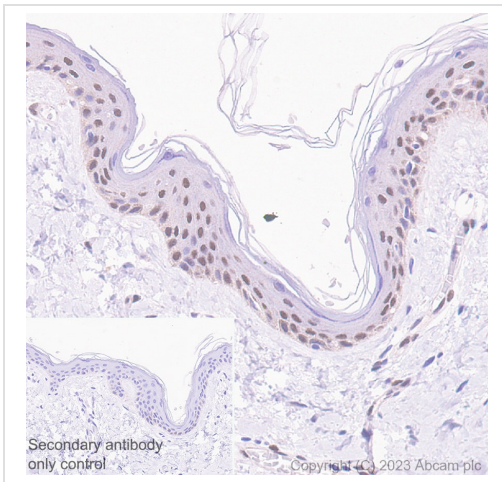


ChIC/CUT&RUN sequencing - Anti-c-Myc antibody [Y69] - ChIP Grade (ab32072)

ChIC/CUT&RUN was performed using a pAG-MNase at a final concentration of 700 ng/μL, 2.5 x 10<sup>5</sup> 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 downloaded [here](#).

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



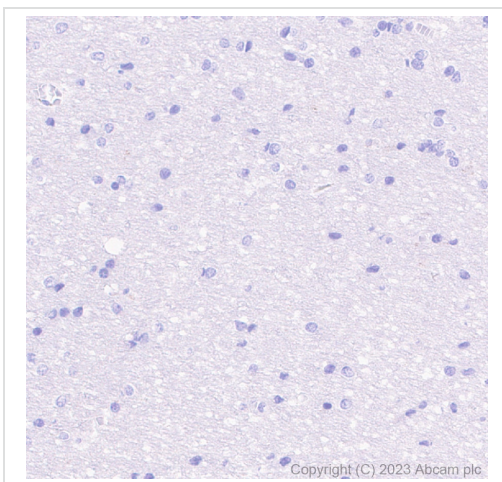
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-c-Myc antibody [Y69] - ChIP Grade (ab32072)

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 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.



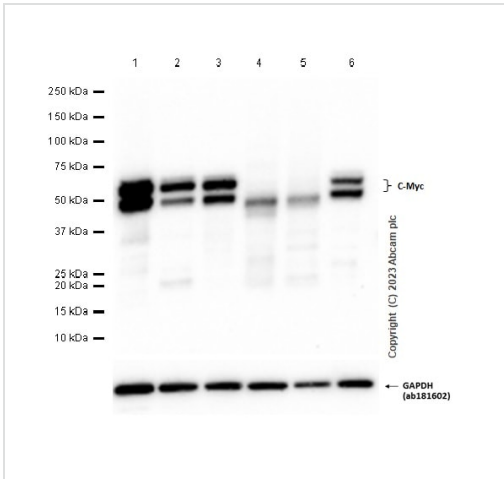
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-c-Myc antibody [Y69] - ChIP Grade (ab32072)

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 ab32072 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 (ab32072)

**All lanes** : Anti-c-Myc antibody [Y69] - ChIP Grade (ab32072) 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

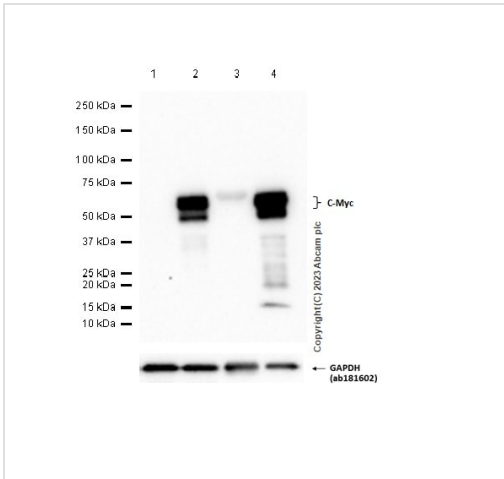
**All lanes** : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

**Predicted band size:** 49 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 (ab32072)

**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**

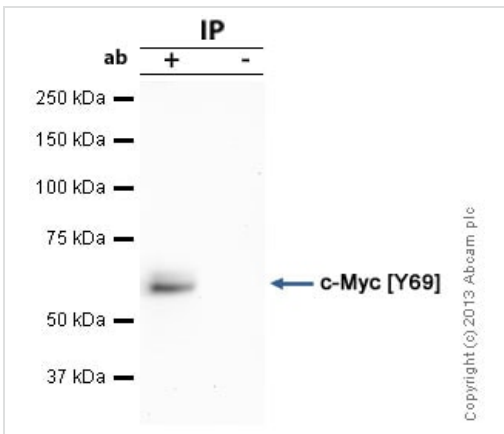
**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

**Predicted band size:** 49 kDa

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

**Exposure time:** 60 seconds

Blocking buffer: 5% NFDM/TBST.



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

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 (-).

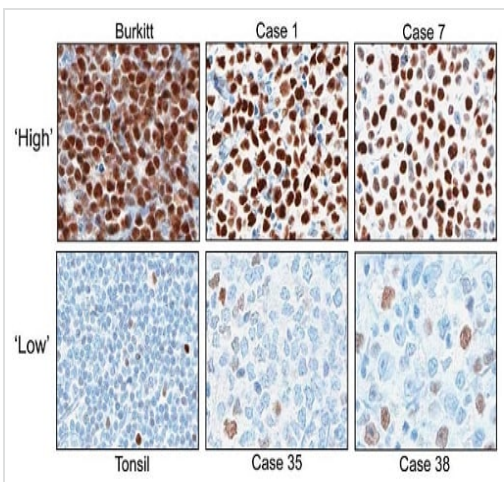
The antibody was incubated under agitation with Protein G beads for 10min, Jurkat whole cell extract lysate diluted in RIPA buffer was added to each sample and incubated for a further 10min under agitation.

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 ab32072.

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

Band: 57kDa; c-Myc [Y69]

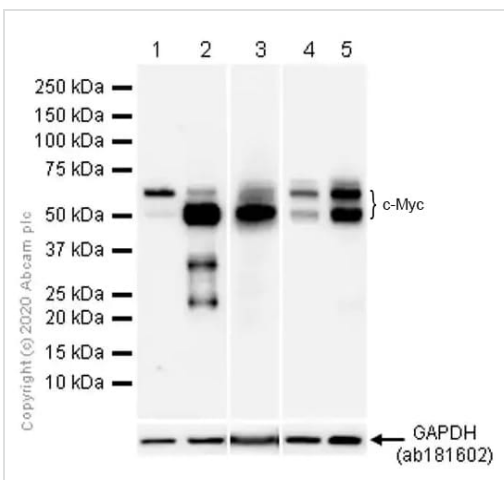




Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-c-Myc antibody [Y69] - ChIP Grade (ab32072)

Image from Kluk MJ et al., PLoS One. 2012;7(4):e33813. Fig 1.; doi: 10.1371/journal.pone.0033813. Reproduced under the Creative Commons license <http://creativecommons.org/licenses/by/4.0/>

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.



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

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

**Lane 1** : Rat pancreas lysates

**Lane 2** : AR42J (Rat pancreatic tumor epithelial cell) whole cell lysates

**Lane 3** : Rat-1 (Rat embryonic fibroblast) whole cell lysates

**Lane 4** : HEK-293 (Human embryonic kidney epithelial cell) whole cell lysates

**Lane 5** : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates

Lysates/proteins at 20 µg per lane.

### Secondary

**All lanes** : Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) at 20000 µg/ml

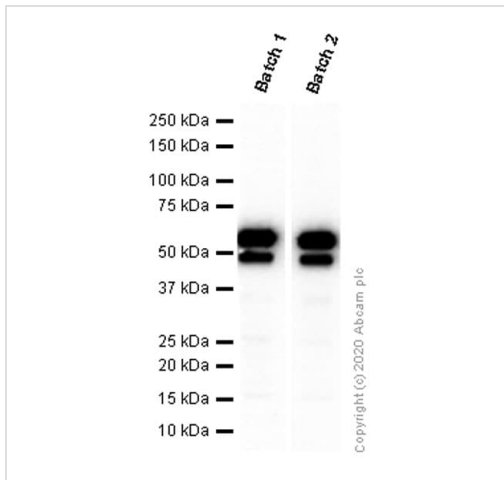
**Predicted band size:** 49 kDa

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

**Blocking/Diluting buffer and concentration:** 5% NFDM/TBST

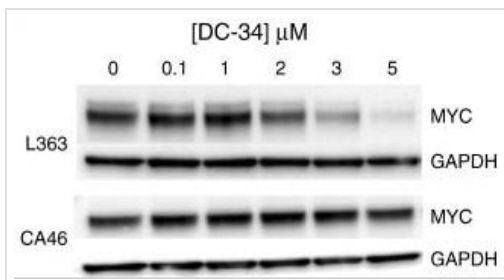
**Lanes 1 and 2:** 80 seconds exposure time

**Lanes 3 to 5:** 5 seconds exposure time



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

Different batches of ab32072 were tested on Raji (Human Burkitt's lymphoma B lymphocyte) whole cell lysate at 0.2  $\mu\text{g/ml}$ . 15  $\mu\text{g}$  of lysate was loaded in each lane. Bands observed at 57 kDa.

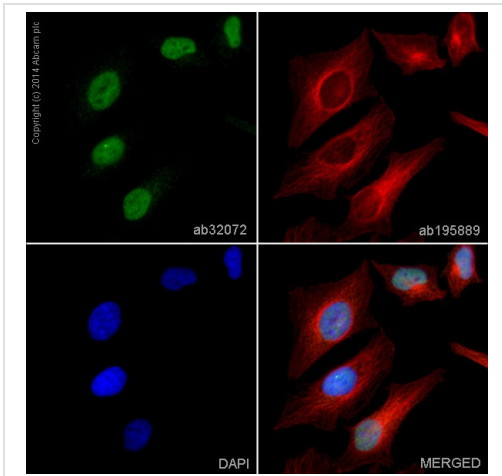


Western blot - Anti-c-Myc antibody [Y69] - ChIP  
Grade (ab32072)

This image is taken from Calabrese D. R. et al. Nat Commun. 2018; 9: 4229. Fig 3e doi: 10.1038/s41467-018-06315-w Reproduced under the Creative Commons license <http://creativecommons.org/licenses/by/4.0/>.

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.

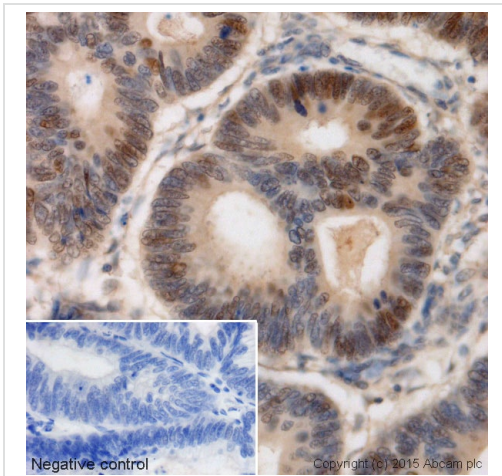
c-Myc protein levels are inhibited as a function of the dose of DC-34 in L363 cells; only the highest dose of DC-34 affected c-Myc in the more resistant CA46 Burkitt's lymphoma cells.



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

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).

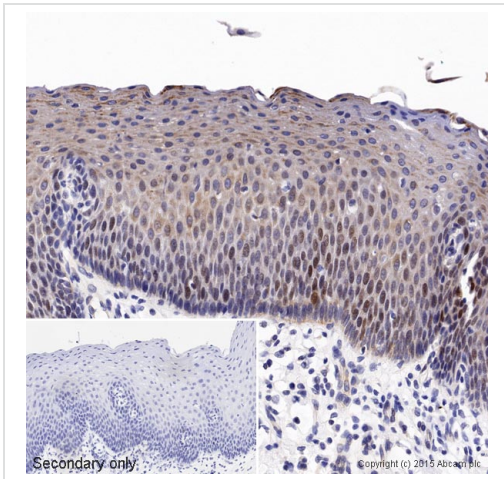
Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



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

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).

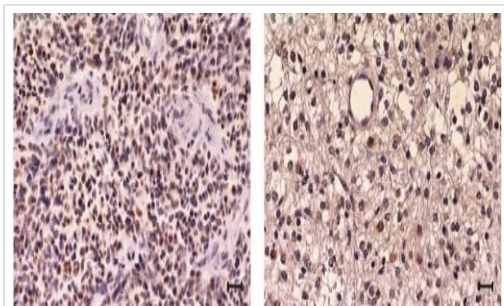
For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.



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

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, 1 µg/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.

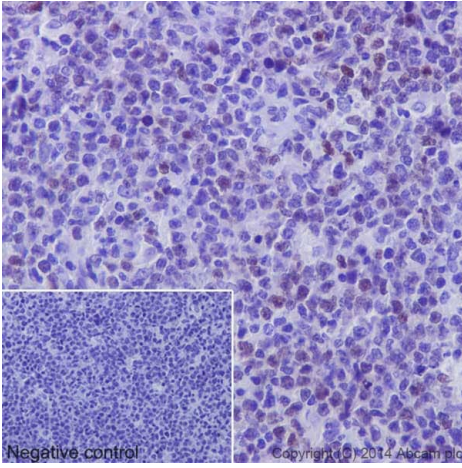


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-c-Myc antibody [Y69] - ChIP Grade (ab32072)

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).

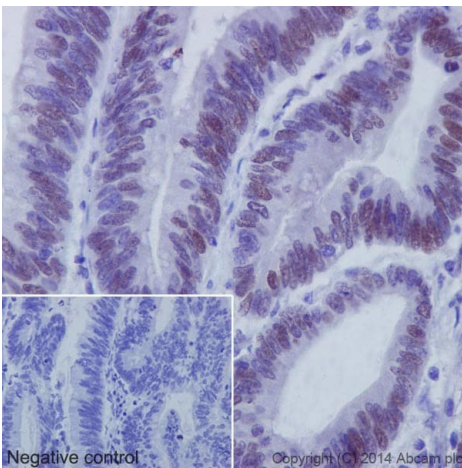
For the full image see PMID 25050814.

Image from Simeone P et al., PLoS One. 2014;;9(7):e103030.; doi: 10.1371/journal.pone.0103030. Reproduced under the Creative Commons license <http://creativecommons.org/licenses/by/4.0/>



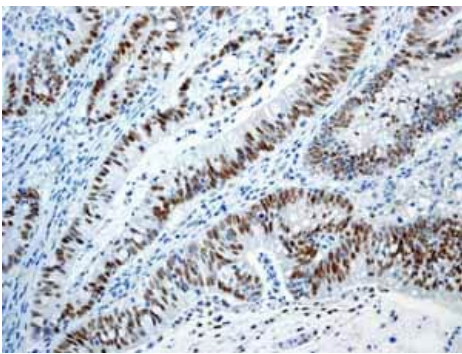
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human diffuse large B cell lymphoma tissue labelling c-Myc with purified ab32072 at 1/500. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. **Goat Anti-Rabbit IgG H&L (HRP) (ab97051) secondary antibody**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

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



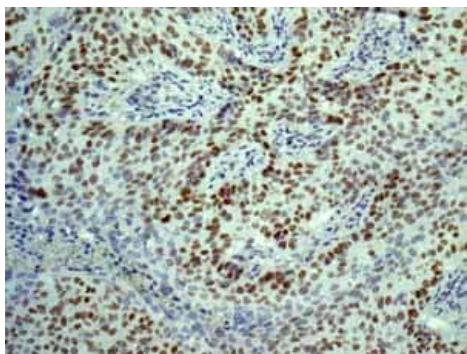
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human adenocarcinoma of the colon tissue labelling c-Myc with purified ab32072 at 1/500. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. **Goat Anti-Rabbit IgG H&L (HRP) (ab97051) secondary antibody**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

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



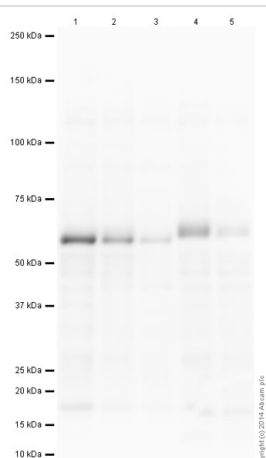
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human adenocarcinoma of colon tissue labelling c-Myc with unpurified ab32072.

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



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

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



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

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

**Lane 1** : Raji (Human Burkitt's lymphoma cell line) Whole Cell Lysate

**Lane 2** : K562 (Human erythromyeloblastoid leukemia cell line) Whole Cell Lysate

**Lane 3** : THP1 (Human acute monocytic leukemia cell line) Whole Cell Lysate

**Lane 4** : A20 (Mouse B lymphoma cell line) Whole Cell Lysate

**Lane 5** : RAW 264.7 (Mouse leukaemic monocyte macrophage cell line) Whole Cell Lysate

Lysates/proteins at 20 µg per lane.

### Secondary

**All lanes** : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/50000 dilution

Developed using the ECL technique.

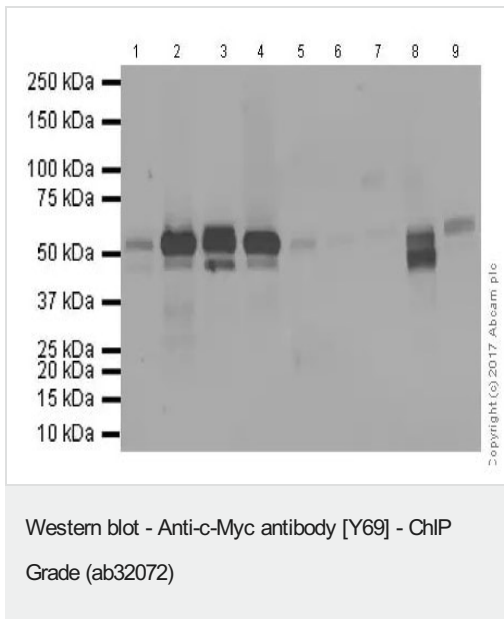
Performed under reducing conditions.

**Predicted band size:** 49 kDa

**Observed band size:** 57 kDa

The predicted molecular weight of c-Myc is 48 kDa (SwissProt), however we expect to observe a banding pattern at 57 kDa.

This blot was produced using a 4-12% Bis-tris gel under the MOPS buffer system. The gel was run at 200V for 50 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using 2% Bovine Serum Albumin before being incubated with ab32072 overnight at 4°C. Antibody binding was detected using an anti-rabbit HRP antibody, and visualised using ECL development solution **ab133406**.



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

**Lane 1** : MCF-7 (Human breast adenocarcinoma epithelial cell) whole cell lysates

**Lane 2** : Raji (Human Burkitt's lymphoma B lymphocyte) whole cell lysates

**Lane 3** : K562 (Human chronic myelogenous leukemia lymphoblast) whole cell lysates

**Lane 4** : Jurkat (Human T cell leukemia T lymphocyte) whole cell lysates

**Lane 5** : THP-1 (Human monocytic leukemia monocyte) whole cell lysates

**Lane 6** : Rat spleen whole cell lysates

**Lane 7** : L6 (Rat skeletal muscle myoblast) whole cell lysates

**Lane 8** : Neuro-2a (Mouse neuroblastoma neuroblast) whole cell lysates

**Lane 9** : RAW264.7 (Mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysates

Lysates/proteins at 20 µg per lane.

### Secondary

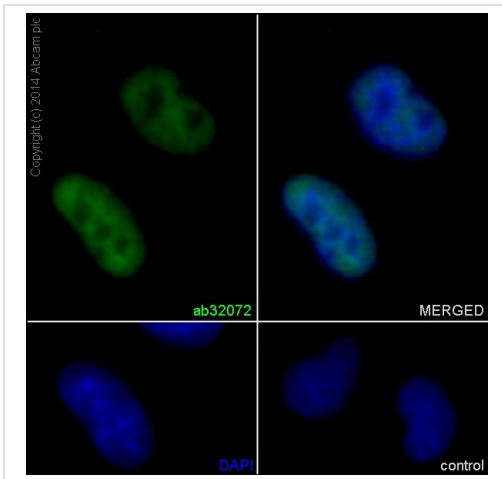
**All lanes** : Peroxidase-conjugated goat anti-rabbit IgG (H+L) at 1/20000 dilution

**Predicted band size:** 49 kDa

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

**Exposure time:** 3 minutes

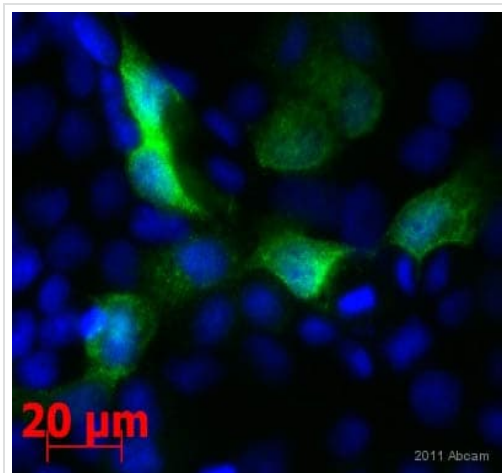
**Blocking and dilution buffer:** 5% NFD/MBST.



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

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, **Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) preadsorbed (ab150120)** (1/500).

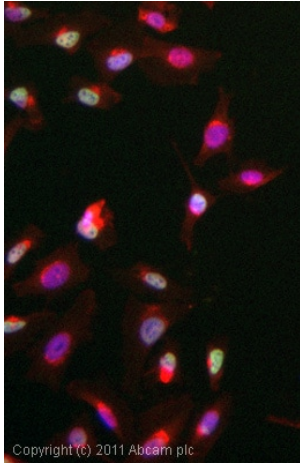


Immunocytochemistry/ Immunofluorescence - Anti-c-Myc antibody [Y69] - ChIP Grade (ab32072)

Image courtesy of Dr Madimir Mlenkovic by Abreview.

Unpurified ab32072 staining c-Myc in HEK293 cells transfected with CACNB4-c-Myc by immunocytochemistry/immunofluorescence. Cells were fixed in paraformaldehyde, permeabilized with 0.5% Triton X-100 then blocked using 5% serum for 20 minutes at 25°C. Samples were then incubated with ab32072 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.

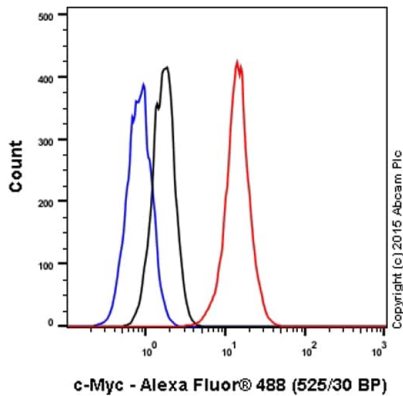




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Immunocytochemistry/ Immunofluorescence - Anti-c-Myc antibody [Y69] - ChIP Grade (ab32072)

ICC/IF image of unpurified ab32072 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 (ab32072, 1µg/ml) overnight at +4°C. The secondary antibody (green) was **Goat Anti-Rabbit IgG H&L (DyLight® 488) preadsorbed (ab96899)** 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.

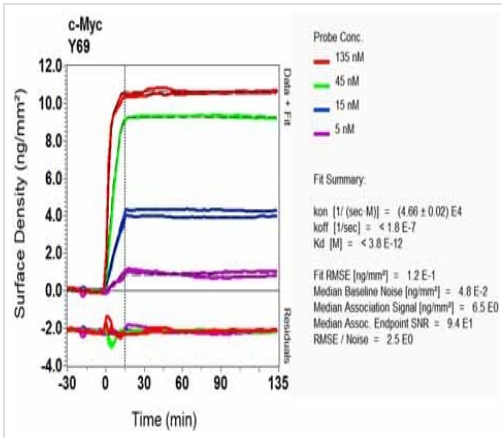


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Flow Cytometry (Intracellular) - Anti-c-Myc antibody [Y69] - ChIP Grade (ab32072)

Overlay histogram showing HeLa (human epithelial cell line from cervix adenocarcinoma) cells stained with ab32072 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab32072, 1/76 dilution) for 30 min at 22°C. The secondary antibody used was Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed (**ab150081**) secondary antibody at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG [EPR25A] (monoclonal) (**ab172730**, 1µg/1x10<sup>6</sup> 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.

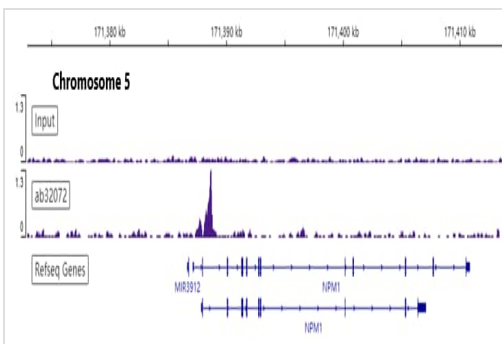


Ox-LD Scanning - Anti-c-Myc antibody [Y69] - ChIP Grade (ab32072)

Equilibrium disassociation constant ( $K_D$ )

Learn more about  $K_D$

[Click here to learn more about  \$K\_D\$](#)







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  $10^7$  NCCIT cells and  $8\mu\text{g}$  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](#).*

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 <p><b>Success from the first experiment</b> Confirmed specificity</p>	 <p><b>Ethical standards compliant</b> Animal-free production</p>

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

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