Anti-c-Myc antibody [Y69] ab32072

Overview

Product name: Anti-c-Myc antibody [Y69]
Description: Rabbit monoclonal [Y69] to c-Myc
Host species: Rabbit
Specificity: This antibody is specific for endogenous c-Myc. Expression levels of the target protein vary with sample type and some optimisation may be required.
Tested applications: Suitable for: WB, ICC/IF, Flow Cyt, IHC-P, IP
Species reactivity: Reacts with: Mouse, Rat, Human
Immunogen: Synthetic peptide within Human c-Myc aa 1-100 (N terminal). The exact sequence is proprietary. (Peptide available as ab166837)
Positive control: Purchase matching WB positive control: Recombinant human c-Myc protein

General notes
Myc is involved in MAPK-p38 signaling pathway - see the interactive version.

If you need conjugated anti-c-myc (Y69) RabMAb antibodies, find our range of products here.
We also offer a PBS only version of this clone as product ab168727.
For more information on choosing the right c-Myc antibody for you, please visit the following webpage.
Abcam recommended secondaries - Goat Anti-Rabbit HRP (ab205718) and Goat Anti-Rabbit Alexa Fluor® 488 (ab150077).
See other anti-rabbit secondary antibodies that can be used with this antibody.
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.
This product is a recombinant rabbit monoclonal antibody.
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 ($K_D$)

$$K_D = 3.80 \times 10^{-12} \text{ M}$$

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

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.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>WB</td>
<td>★★★★★</td>
<td>1/1000. Detects a band of approximately 57 kDa (predicted molecular weight: 49 kDa). Can be blocked with Human c-Myc peptide (ab166837).</td>
</tr>
<tr>
<td>ICC/IF</td>
<td>★★★★★</td>
<td>Use a concentration of 10 µg/ml. 1/100.</td>
</tr>
<tr>
<td>Flow Cyt</td>
<td></td>
<td>1/76.</td>
</tr>
<tr>
<td>IHC-P</td>
<td>★★★★☆</td>
<td>Use a concentration of 5 µg/ml. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.</td>
</tr>
<tr>
<td>IP</td>
<td>★★★★☆</td>
<td>Use a concentration of 5 µg/ml.</td>
</tr>
</tbody>
</table>

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. A chromosomal aberration involving MYC may be a cause 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

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.
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.

**Western blot**

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

Lane 1: Raji (Human Burkitt’s lymphoma cell line) Whole Cell Lysate
Lane 2: K562 (Human erythroleukemia 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

*why is the actual band size different from the predicted?*

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.

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]

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] (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, 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.

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. The isotype control antibody (black line) was rabbit IgG [EPR25A] (monoclonal) (ab172730, 1μg/1x10^6 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.
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.

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] (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.

Immunocytochemistry/immunofluorescence - Anti-c-Myc antibody [Y69] (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).
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.

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.

**All lanes**: Anti-c-Myc antibody [Y69] (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: 57 kDa why is the actual band size different from the predicted?

Exposure time: 3 minutes

Blocking and dilution buffer: 5% NFDM/TBST.

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) 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) analysis of human skin carcinoma tissue labelling c-Myc with unpurified ab32072 at 1/50.
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) analysis of human lung adenocarcinoma tissue labelling c-Myc with unpurified ab32072.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human gastric adenocarcinoma tissue labelling c-Myc with unpurified ab32072.
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human urinary bladder transitional carcinoma tissue labelling c-Myc with unpurified ab32072.

Equilibrium disassociation constant ($K_D$)

Learn more about $K_D$

Click here to learn more about $K_D$

Please note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE"

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