abcam

Product datasheet

Anti-c-Myc (phospho T58) antibody [EPR17923] ab185655

Recombinant RabMAb

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Overview

Product name	Anti-c-Myc (phospho T58) antibody [EPR17923]
Description	Rabbit monoclonal [EPR17923] to c-Myc (phospho T58)
Host species	Rabbit
Tested applications	**Suitable for:** ICC/IF, WB, Flow Cyt (Intra), Dot blot
Species reactivity	**Reacts with:** Human **Predicted to work with:** Mouse, Rat
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysate, HeLa cells treated with 200nM Calyculin A and 1uM Okadaic Acid for 60 minutes whole cell lysate. ICC/IF: HeLa cells. Flow Cyt (intra): HeLa cells
General notes	This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified

Clonality	Monoclonal
Clone number	EPR17923
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab185655 in the following tested applications.

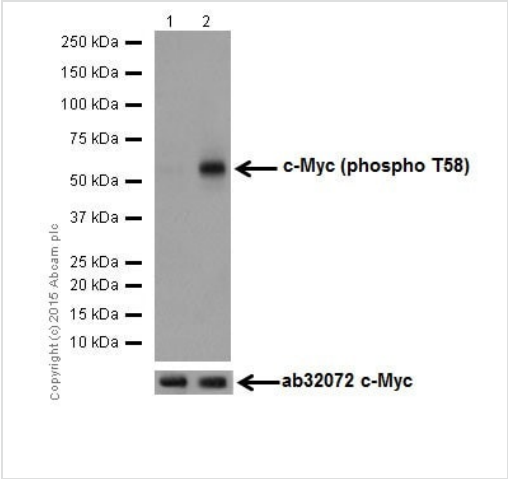
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ICC/IF		1/250.
WB	★★★★☆ (1)	1/1000. Detects a band of approximately 57 kDa (predicted molecular weight: 49 kDa).
Flow Cyt (Intra)		1/700.
Dot blot		1/1000.

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



Western blot - Anti-c-Myc (phospho T58) antibody [EPR17923] (ab185655)

All lanes : Anti-c-Myc (phospho T58) antibody [EPR17923] (ab185655) at 1/5000 dilution

Lane 1 : Untreated HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 2 : HeLa (Human epithelial cell line from cervix adenocarcinoma) treated with 200nM Calyculin A and 1uM Okadaic Acid for 60 minutes whole cell lysate

Lysates/proteins at 10 µg per lane.

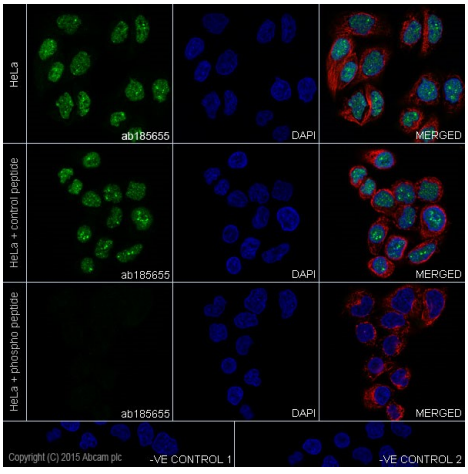
Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L),Peroxidase conjugated at 1/1000 dilution

Developed using the ECL technique.

Predicted band size: 49 kDa
Observed band size: 57 kDa

Exposure time: 3 minutes

Blocking and diluting buffer was 5% NFDM /TBST



Immunocytochemistry/ Immunofluorescence - Anti-c-Myc (phospho T58) antibody [EPR17923] (ab185655)

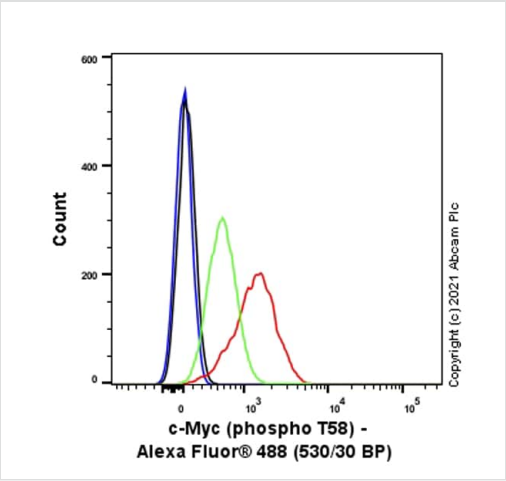
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa cells (Human epithelial cells from cervix adenocarcinoma) labeling c-Myc (phospho T58) with ab185655 at 1/250, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/500 (green).

Confocal image showing nuclear staining on HeLa cells. The staining decreased after blocking with phospho peptide (100µg/ml) overnight. The control peptide is a non-phospho peptide.

The nuclear counterstain is DAPI (blue).

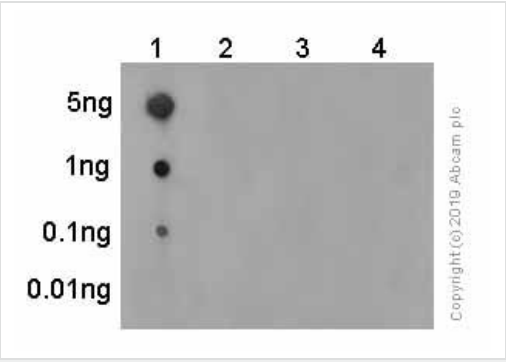
Tubulin is detected with Anti-alpha Tubulin antibody -Loading Control (**ab7291**) at 1/1000 dilution Goat Anti-Mouse IgG (AlexaFluor®594) preadsorbed (**ab150120**) at 1/500 (red).

The negative controls are as follows:-
-ve control 1 - ab185655 at 1/500 followed by **ab150120** at 1/500.
-ve control 2 -**ab7291** at 1/1000 followed by **ab150077** at 1/500.



Flow Cytometry (Intracellular) - Anti-c-Myc (phospho T58) antibody [EPR17923] (ab185655)

Flow Cytometry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) treated with 200nM Calyculin A and 1uM Okadaic Acid for 60 min cells labeling c-Myc with purified ab185655 at 1/700 dilution (1 µg/mL) (Red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) secondary antibody was used at 1/2000. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).



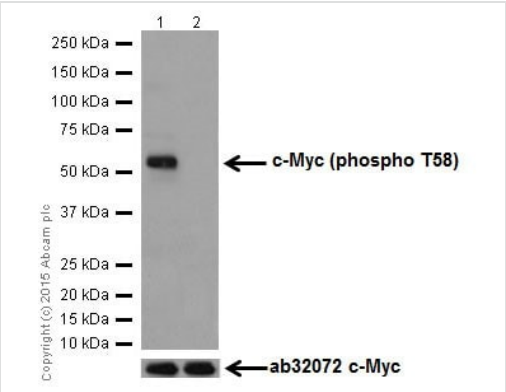
Dot Blot - Anti-c-Myc (phospho T58) antibody [EPR17923] (ab185655)

Lane 1: c-Myc (phospho T58).
Lane 2: c-Myc (pT58) non-phospho peptide.
Lane 3: c-Myc (pS62) phospho peptide.
Lane 4: c-Myc (pS62) non-phospho peptide.

Dot blot analysis using ab185655 at a dilution of 1/1000. Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (**ab97051**) was used as the secondary antibody at a dilution of 1/100000.

Blocking and dilution buffer: 5% NFDM/TBST.

Exposure time: 3 minutes.



Western blot - Anti-c-Myc (phospho T58) antibody [EPR17923] (ab185655)

All lanes : Anti-c-Myc (phospho T58) antibody [EPR17923] (ab185655) at 1/1000 dilution

Lane 1 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 2 : HeLa (Human epithelial cell line from cervix adenocarcinoma) treated with Lambda Phosphatase whole cell lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L),Peroxidase conjugated at 1/1000 dilution

Developed using the ECL technique.

Predicted band size: 49 kDa

Observed band size: 57 kDa

Exposure time: 3 minutes

Blocking and diluting buffer was 5% NFDM /TBST.

The strong band in Lane 1 of WB-2 compared to WB-1 is due to different lysate batches and a lower dilution factor (1:1000).

Anti-c-Myc (phospho T58) antibody [EPR17923] (ab185655)

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