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.

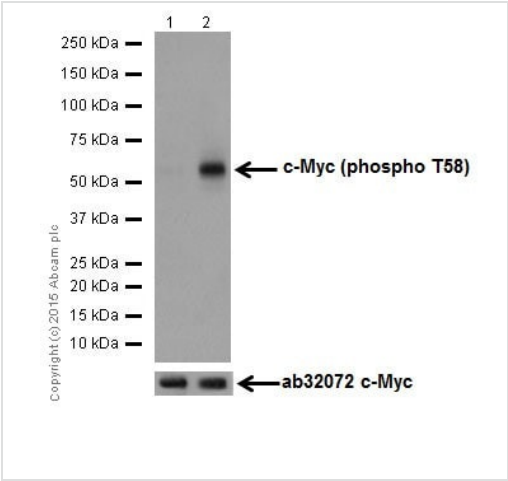
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

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.

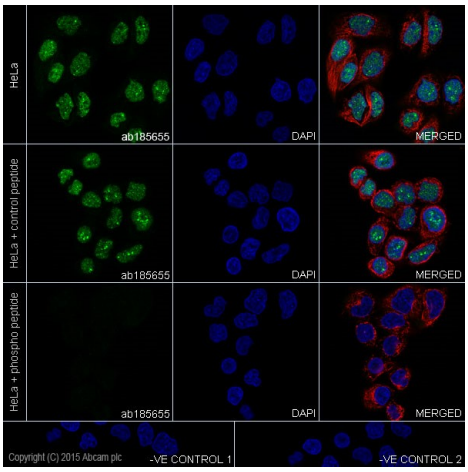
Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L),Peroxidase conjugated at 1/1000 dilution

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)

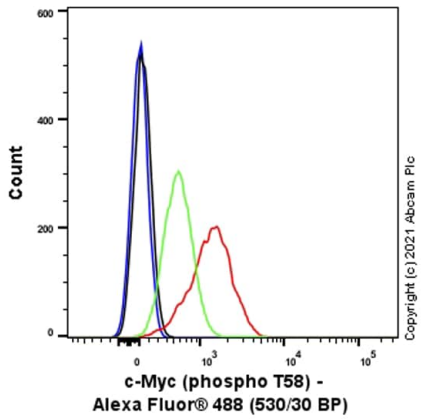
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa cells (Human epithelial cells from cervix adenocarcinoma) labeling c-Myc (phospho T58) with ab185655 at 1/250, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/500 (green).

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).

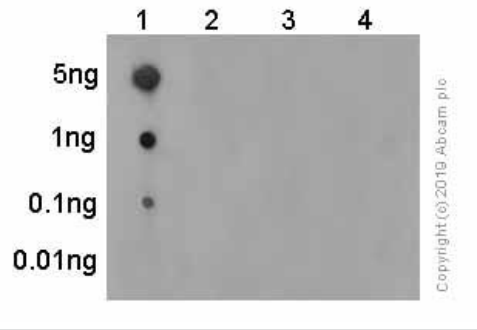
Tubulin is detected with Anti-alpha Tubulin antibody -Loading Control (ab7291) at 1/1000 dilution Goat Anti-Mouse IgG (AlexaFluor®594) preadsorbed (ab150120) at 1/500 (red).

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).



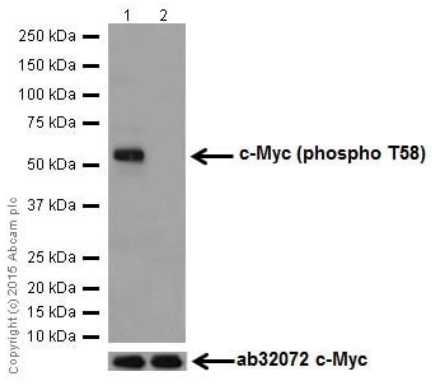
Dot Blot - Anti-c-Myc (phospho T58) antibody [EPR17923] (ab185655)

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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