abcam

Product datasheet

Anti-LEF1 antibody [EP2030Y] - BSA and Azide free ab227562

KO VALIDATED Recombinant RabMAb

6 References 8 Images

Overview

Product name	Anti-LEF1 antibody [EP2030Y] - BSA and Azide free
Description	Rabbit monoclonal [EP2030Y] to LEF1 - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, IHC-P, ICC/IF
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	Jurkat cell lysate and human colon adenocarcinoma tissue.
General notes	ab227562 is the carrier-free version of **ab53293**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. 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**. Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with

these species. Please contact us for more information.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.20 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP2030Y
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab227562 in the following tested applications.

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

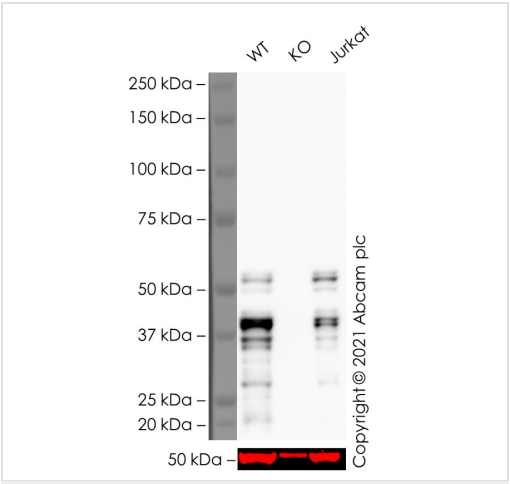
Application	Abreviews	Notes
Flow Cyt (Intra)		Use at an assay dependent concentration. **ab199376** - Rabbit monoclonal IgG (Low endotoxin, Azide free), is suitable for use as an isotype control with this antibody.
WB		Use at an assay dependent concentration. Detects a band of approximately 55 kDa (predicted molecular weight: 44 kDa).
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**.
ICC/IF		Use at an assay dependent concentration.

Target

Function Participates in the Wnt signaling pathway. Activates transcription of target genes in the presence of CTNNB1 and EP300. May play a role in hair cell differentiation and follicle morphogenesis. TLE1, TLE2, TLE3 and TLE4 repress transactivation mediated by LEF1 and CTNNB1. Regulates T-cell receptor alpha enhancer function. Binds DNA in a sequence-specific manner. PIAG antagonizes both Wnt-dependent and Wnt-independent activation by LEF1 (By similarity). Isoform 3 lacks the CTNNB1 interaction domain and may be an antagonist for Wnt signaling. Isoform 5 transcriptionally activates the fibronectin promoter, binds to and represses transcription from the E-cadherin promoter in a CTNNB1-independent manner, and is involved in reducing cellular aggregation and increasing cell migration of pancreatic cancer cells. Isoform 1 transcriptionally

	activates MYC and CCND1 expression and enhances proliferation of pancreatic tumor cells.
Tissue specificity	Detected in thymus. Not detected in normal colon, but highly expressed in colon cancer biopsies and colon cancer cell lines. Expressed in several pancreatic tumors and weakly expressed in normal pancreatic tissue. Isoforms 1 and 5 are detected in several pancreatic cell lines.
Sequence similarities	Belongs to the TCF/LEF family. Contains 1 HMG box DNA-binding domain.
Domain	Proline-rich and acidic regions are implicated in the activation functions of RNA polymerase II transcription factors.
Cellular localization	Nucleus. Found in nuclear bodies upon PIASG binding.

Images



Western blot - Anti-LEF1 antibody [EP2030Y] - BSA and Azide free (ab227562)

All lanes : Anti-LEF1 antibody [EP2030Y] (**ab53293**) at 1/5000 dilution

Lane 1 : Wild-type Jurkat cell lysate at 40 µg
Lane 2 : Lef1 knockout Jurkat cell lysate at 40 µg
Lane 3 : Jurkat cell lysate at 20 µg

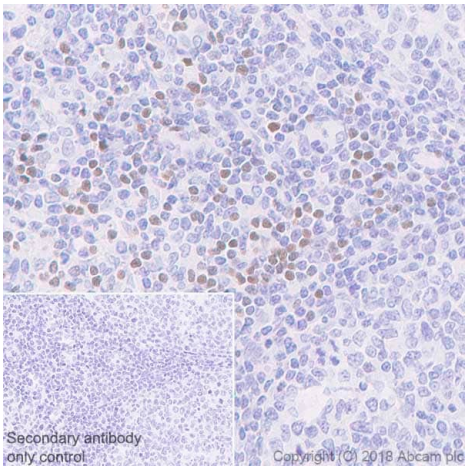
Performed under reducing conditions.

Predicted band size: 44 kDa
Observed band size: 40 kDa

False colour image of Western blot: Anti-LEF1 antibody [EP2030Y] staining at 1/5000 dilution, shown in black; Mouse anti-Alpha Tubulin [DM1A] (**ab7291**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab53293** was shown to bind specifically to LEF1. A band was observed at 40/53 kDa in wild-type Jurkat cell lysates with no signal observed at this size in Lef1 knockout cell line **ab274898** (knockout cell lysate **ab274956**). To generate this image, wild-type and Lef1 knockout Jurkat cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % 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 before development with Optiblot (ECL reagent **ab133456**) and imaged with 8 minutes exposure time. Secondary antibodies used were HRP conjugated Goat anti-Rabbit (H+L) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) at 1/20000

dilution.

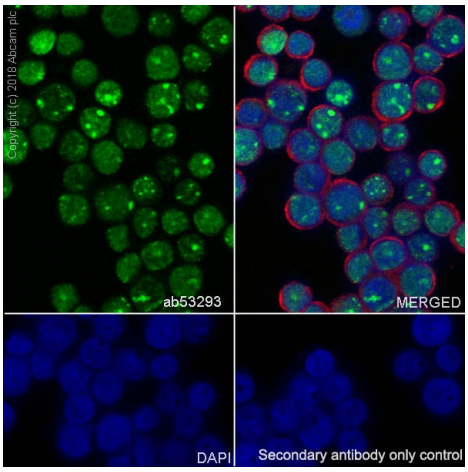
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab53293**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-LEF1 antibody [EP2030Y] - BSA and Azide free (ab227562)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human tonsil tissue sections labeling LEF1 with purified **ab53293** at 1/50 dilution (1.94 µg/ml). Heat mediated antigen retrieval was performed using **ab93684** (Tris/EDTA buffer, pH 9.0). ImmunoHistoProbe one step HRP Polymer (ready to use) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.

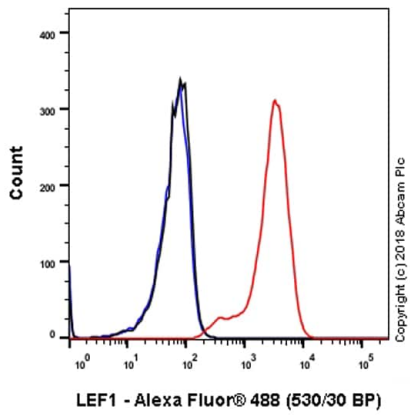
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab53293**).



Immunocytochemistry/ Immunofluorescence - Anti-LEF1 antibody [EP2030Y] - BSA and Azide free (ab227562)

Immunocytochemistry/ Immunofluorescence analysis of Jurkat (Human T cell leukemia T lymphocyte) cells labeling LEF1 with purified **ab53293** at 1/50 dilution (2.0 µg/ml). Cells were fixed in 100% Methanol. Cells were counterstained with **ab195889** Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) at 1/200 (2.5 µg/ml). Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) was used as the secondary antibody at 1/1000 (2 µg/ml) dilution. DAPI (blue) was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.

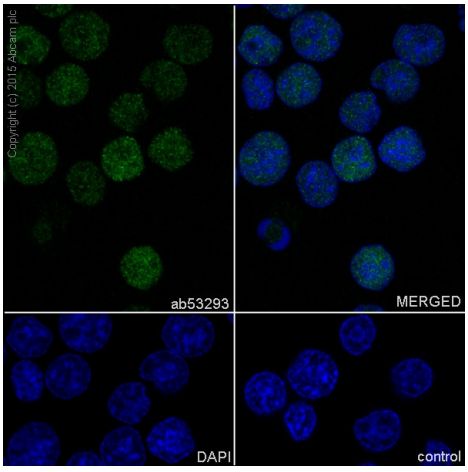
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab53293**).



Flow Cytometry (Intracellular) - Anti-LEF1 antibody [EP2030Y] - BSA and Azide free (ab227562)

Intracellular Flow Cytometry analysis of Jurkat (Human T cell leukemia T lymphocyte) cells labeling LEF1 with purified **ab53293** at 1/20 dilution (10µg/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).

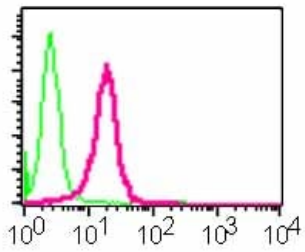
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab53293**).



Immunocytochemistry/ Immunofluorescence - Anti-LEF1 antibody [EP2030Y] - BSA and Azide free (ab227562)

ab53293 (unpurified) staining LEF1 in RAMOS (human Burkitt's lymphoma) cells by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. Samples were incubated with primary antibody at a dilution of 1/500. A goat anti rabbit IgG (Alexa Fluor® 488) (**ab150077**) was used as the secondary antibody at a dilution of 1/1000. DAPI was used as a nuclear counterstain and the negative control was PBS only.

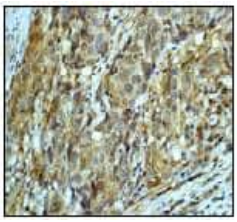
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab53293**).



Flow Cytometry (Intracellular) - Anti-LEF1 antibody [EP2030Y] - BSA and Azide free (ab227562)

Intracellular flow cytometric analysis of permeabilized Jurkat cells using **ab53293** (unpurified) (red) or a rabbit IgG (negative) (green).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab53293**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-LEF1 antibody [EP2030Y] - BSA and Azide free (ab227562)

This IHC data was generated using the same anti-LEF1 antibody clone, EP2030Y, in a different buffer formulation (cat# **ab53293**).

Ab53293 (unpurified) (1:50) staining human LEF1 in human colon adenocarcinoma tissue by immunohistochemistry using paraffin embedded tissue.

Anti-LEF1 antibody [EP2030Y] - BSA and Azide free (ab227562)

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