abcam

Product datasheet

Anti-LEF1 antibody [EP2030Y] ab53293





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Overview

Product name Anti-LEF1 antibody [EP2030Y]

Description Rabbit monoclonal [EP2030Y] to LEF1

Host species Rabbit

Tested applications Suitable for: ICC/IF, Flow Cyt (Intra), WB, IHC-P

Species reactivity Reacts with: Human

Immunogen Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control ICC/IF: Ramos and Jurkat cells. IHC-P: Human colon adenocarcinoma, human tonsil tissue. Flow

Cyt (intra): Jurkat cells. WB: Jurkat cell lysate

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**.

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 short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long

term. Avoid freeze / thaw cycle.

Storage buffer pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

Purity Protein A purified

Clonality Monoclonal

Clone number EP2030Y

Isotype IgG

Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab53293 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		1/50 - 1/250.
Flow Cyt (Intra)		1/20. ab172730 - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
WB		1/5000. Detects a band of approximately 55 kDa (predicted molecular weight: 44 kDa). For unpurified format use at 1/1000 dilution.
IHC-P	★★★☆☆☆(1)	1/50. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. See IHC antigen retrieval protocols .

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

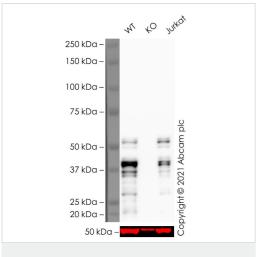
 $\label{proline-rich and acidic regions are implicated in the activation functions of RNA polymerase \ II$

transcription factors.

Cellular localization

Nucleus. Found in nuclear bodies upon PIASG binding.

Images



Western blot - Anti-LEF1 antibody [EP2030Y] (ab53293)

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

Lane 1 : Wild-type Jurkat cell lysate at 40 μg

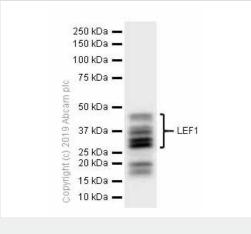
Lane 2: Lef1 knockout Jurkat cell lysate at 40 µg

Lane 3 : Jurkat cell lysate at 20 μg

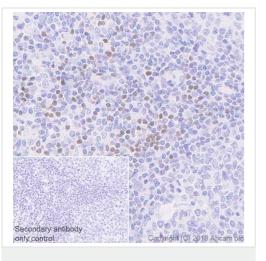
Performed under reducing conditions.

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 wildtype 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.



Western blot - Anti-LEF1 antibody [EP2030Y] (ab53293)



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-LEF1 antibody
[EP2030Y] (ab53293)

Anti-LEF1 antibody [EP2030Y] (ab53293) at 1/5000 dilution + Jurkat (Human T cell leukemia T lymphocyte) whole cell lysates at $15~\mu g$

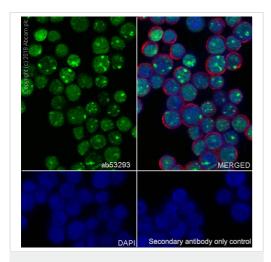
Secondary

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

Predicted band size: 44 kDa **Observed band size:** 23-44 kDa

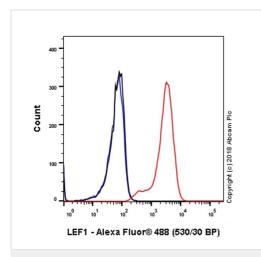
This antibody recognizes all 7 LEF1 isoforms with the MW ranging from 23 to 44 KD based on immunogen blast.

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 heat mediated antigen retrieval 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.



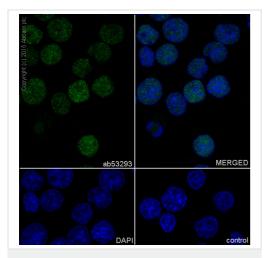
Immunocytochemistry/ Immunofluorescence - Anti-LEF1 antibody [EP2030Y] (ab53293)

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 <u>ab195889</u> Antialpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor[®] 594) at 1:200 (2.5 μg/ml). Goat anti rabbit lgG (Alexa Fluor[®] 488, <u>ab150077</u>) 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.



Flow Cytometry (Intracellular) - Anti-LEF1 antibody [EP2030Y] (ab53293)

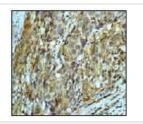
Intracellular Flow Cytometry analysis of Jurkat (Human T cell leukemia T lymphocyte) cells labeling LEF1 with purified ab53293 at 1/20 dilution (10µg/ml) (red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit lgG (Alexa Fluorr® 488, **ab150077**) secondary antibody was used at 1/2000. Isotype control - Rabbit monoclonal lgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).



Immunocytochemistry/ Immunofluorescence - Anti-LEF1 antibody [EP2030Y] (ab53293)

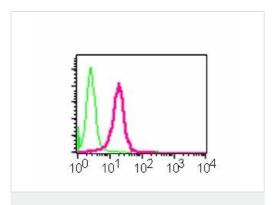
ab53293 (unpurified) staining LEF1 in RAMOS (human Burkitt's lymphoma) cells by ICC/IF

(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.



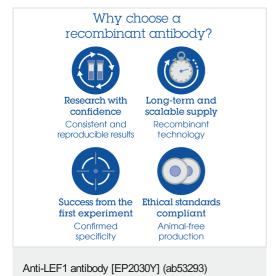
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-LEF1 antibody
[EP2030Y] (ab53293)

Paraffin-embedded human colon adenocarcinoma tissue stained for LEF1 using ab53293 (unpurified) at 1/50 dilution in immunohistochemical analysis.



Flow Cytometry (Intracellular) - Anti-LEF1 antibody [EP2030Y] (ab53293)

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



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