abcam

Product datasheet

Anti-LEF1 antibody [EPR2029Y] - BSA and Azide free ab215999

KO VALIDATED Recombinant RabMAb

3 References 15 Images

Overview

Product name	Anti-LEF1 antibody [EPR2029Y] - BSA and Azide free
Description	Rabbit monoclonal [EPR2029Y] to LEF1 - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** IP, ICC/IF, IHC-P, WB, Flow Cyt (Intra)
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Jurkat cell lysate. IHC-P: Human tonsil tissue. Flow cyto (intra): Jurkat cells
General notes	ab215999 is the carrier-free version of **ab137872**. 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**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR2029Y
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab215999 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
IP		Use at an assay dependent concentration.
ICC/IF		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**.
WB		Use at an assay dependent concentration. Predicted molecular weight: 44 kDa.
Flow Cyt (Intra)		Use at an assay dependent concentration.

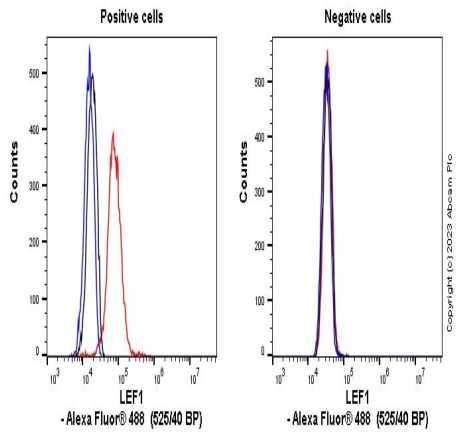
Target

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



Flow Cytometry (Intracellular) - Anti-LEF1 antibody [EPR2029Y] - BSA and Azide free (ab215999)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab137872**).

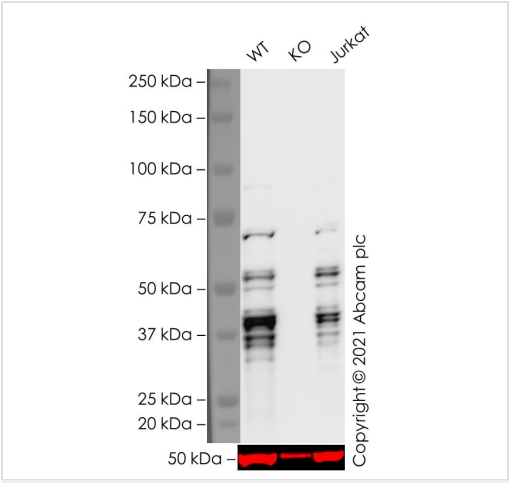
Flow cytometry overlay histogram showing left Jurkat positive cells and right negative HeLa stained with **ab137872** (red line). The cells were fixed with 80% methanol (5 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (**ab137872**) (1x 10^6 in 100µl at 0.2µg/ml (1/11500)) for 30min at 22°C.

The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 for 30min at 22°C

Isotype control antibody (black line) was Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control used at the same concentration and conditions as the primary antibody. Unlabelled sample (blue line) was also used as a control.

Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.

This antibody gave a positive signal in Jurkat Fixed with 4% formaldehyde (10 min) / permeabilised with 0.1% PBS-Triton X-100 for 15 min under the same conditions.



Western blot - Anti-LEF1 antibody [EPR2029Y] - BSA and Azide free (ab215999)

All lanes : Anti-LEF1 antibody [EPR2029Y] (**ab137872**) at 1/1000 dilution

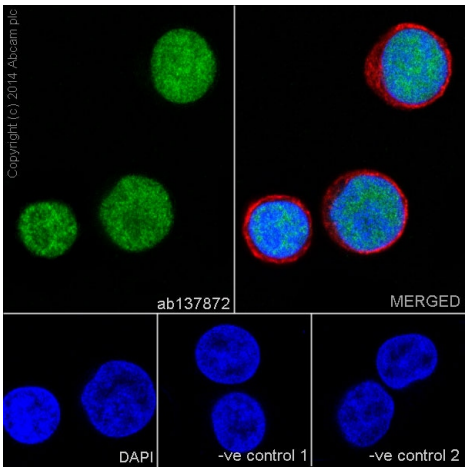
Lane 1 : Wild-type Jurkat cell lysate at 40 µg
Lane 2 : Lef1 knockout Jurkat cell lysate at 40 µg
Lane 3 : Jurkat cell lysate at 20 µg

Performed under reducing conditions.

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

False colour image of Western blot: Anti-LEF1 antibody [EPR2029Y] staining at 1/1000 dilution, shown in black; Mouse anti-Alpha Tubulin [DM1A] (**ab7291**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab137872** 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 4 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 (**ab137872**).

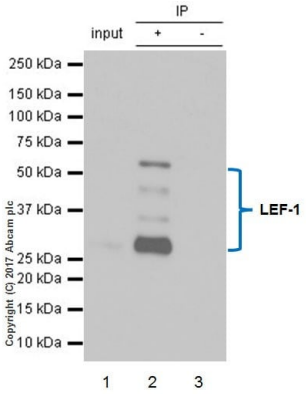


Immunocytochemistry/ Immunofluorescence - Anti-LEF1 antibody [EPR2029Y] - BSA and Azide free (ab215999)

Immunofluorescence staining of Jurkat (Human T cell leukemia cell line from peripheral blood) cells with purified **ab137872** at a working dilution of 1 in 500, counter-stained with DAPI. Tubulin was stained with mouse anti-tubulin at a dilution of 1/1000 (**ab7291**) and Alexa Fluor® 594 goat anti-mouse at a dilution of 1/500 (**ab150120**) . The secondary antibody was **ab150077** Alexa Fluor® 488 goat anti rabbit, used at a dilution of 1 in 500. The cells were fixed in 4% PFA and permeabilized using 0.1% Triton X 100.

The negative controls are shown in the bottom middle and right hand panels - for the first negative control, purified **ab137872** was used at a dilution of 1/200 followed by an Alexa Fluor® 555 goat anti-mouse antibody at a dilution of 1/500 and for the second negative control mouse primary antibody (**ab7291**) and anti-rabbit secondary antibody (**ab15007**) were used.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab137872**).



Immunoprecipitation - Anti-LEF1 antibody [EPR2029Y] - BSA and Azide free (ab215999)

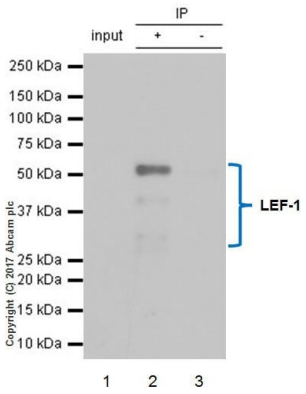
Lane 1 (input): Rat thymus lysate, 10µg
Lane 2 (+): Rat thymus lysate
Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of **ab137872** in rat thymus lysate

ab137872 immunoprecipitating LEF1 in rat thymus lysate. For western blotting, primary antibody used was **ab137872** at 1:1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/10,000 dilution.

Blocking and diluting buffer: 5% NFDM/TBST

Exposure time: 3 minutes

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab137872**).



Immunoprecipitation - Anti-LEF1 antibody [EPR2029Y] - BSA and Azide free (ab215999)

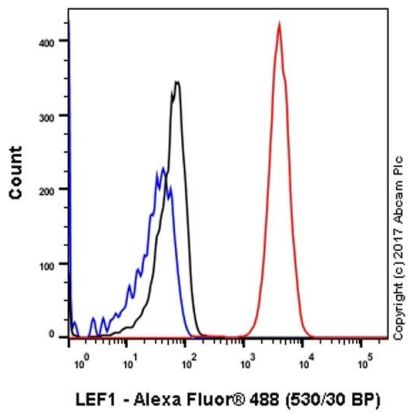
Lane 1 (input): Jurkat (human T cell leukemia T lymphocyte) whole cell lysate, 10 µg
Lane 2 (+): Jurkat whole cell lysate
Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of **ab137872** in Jurkat whole cell lysate

ab137872 immunoprecipitating LEF1 in Jurkat whole cell lysate. For western blotting, primary antibody used was **ab137872** at 1:1000 dilution. **ab131366** VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/10,000 dilution.

Blocking and diluting buffer: 5% NFDM/TBST

Exposure time: 3 minutes

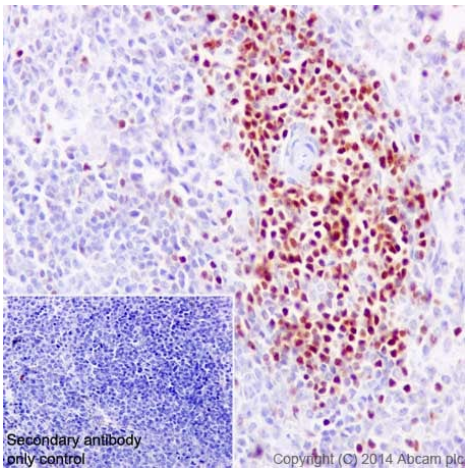
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab137872**).



Flow Cytometry (Intracellular) - Anti-LEF1 antibody [EPR2029Y] - BSA and Azide free (ab215999)

Intracellular flow cytometric analysis of Jurkat cell line (human T cell leukemia T lymphocyte) fixed with 4% paraformaldehyde and permeabilized with 90% methanol labeling LEF1 with **ab137872** at 1/600 dilution (red). This is compared with a Rabbit monoclonal IgG (**ab172730**) - Isotype control (black) and a unlabeled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat anti-rabbit IgG (Alexa Fluor® 488) was used as the secondary antibody.

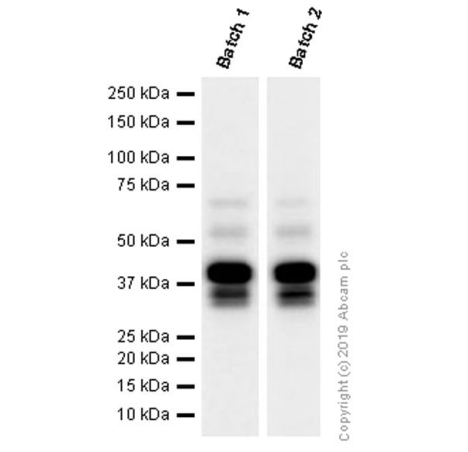
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab137872**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-LEF1 antibody [EPR2029Y] - BSA and Azide free (ab215999)

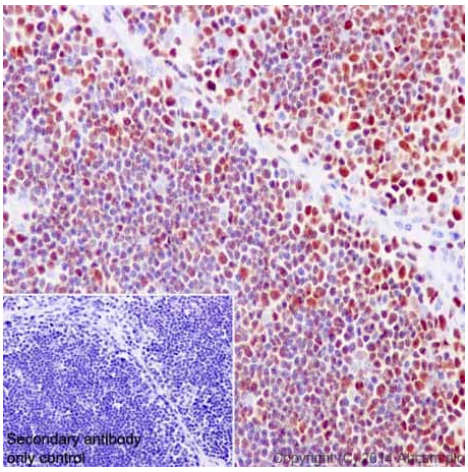
Immunohistochemical staining of paraffin embedded rat spleen with purified **ab137872** at a working dilution of 1/500. The secondary antibody used is **ab97051**, an HRP-conjugated goat anti-rabbit IgG (H+L), at a dilution of 1/500. The sample is counter-stained with hematoxylin. Antigen retrieval was perfomed using Tris-EDTA buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab137872**).



Western blot - Anti-LEF1 antibody [EPR2029Y] - BSA and Azide free (ab215999)

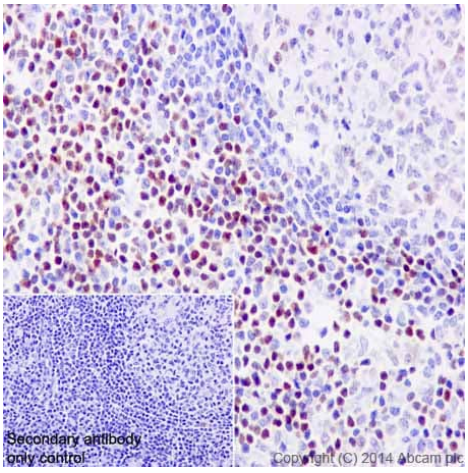
This data was developed using **ab137872**, the same antibody clone in a different buffer formulation. Different batches of **ab137872** were tested on Jurkat (Human T cell leukemia T lymphocyte) lysate at 1.1 µg/ml. 15 µg of lysate was loaded in each lane. Bands observed at 25-57 kDa.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-LEF1 antibody [EPR2029Y] - BSA and Azide free (ab215999)

Immunohistochemical staining of paraffin-embedded human thymus with purified **ab137872** at a working dilution of 1/500. The secondary antibody used is **ab97051**, an HRP-conjugated goat anti-rabbit IgG (H+L), at a dilution of 1/500. The sample is counter-stained with hematoxylin. Antigen retrieval was perfomed using Tris-EDTA buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.

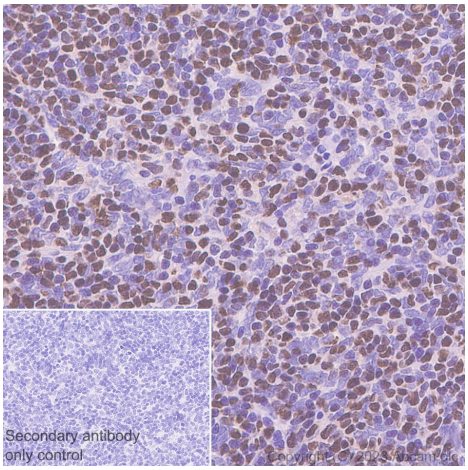
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab137872**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-LEF1 antibody [EPR2029Y] - BSA and Azide free (ab215999)

Immunohistochemical staining of paraffin embedded human tonsil with purified **ab137872** at a working dilution of 1/500. The secondary antibody used is **ab97051**, an HRP-conjugated goat anti-rabbit IgG (H+L), at a dilution of 1/500. The sample is counter-stained with hematoxylin. Antigen retrieval was perfomed using Tris-EDTA buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.

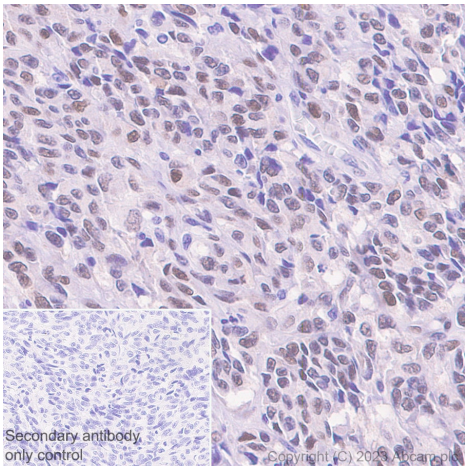
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab137872**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-LEF1 antibody [EPR2029Y] - BSA and Azide free (ab215999)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab137872**).

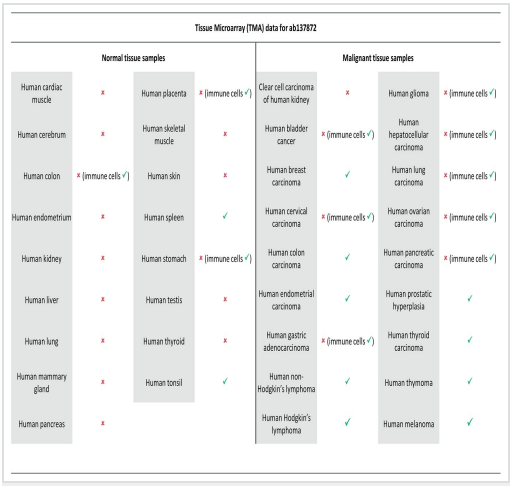
ab137872 staining LEF1 in paraffin embedded human thymona tissue by Immunohistochemistry. Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins. Samples were incubated with primary antibody at 1/2000 dilution for 30 mins at room temperature. A ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) was used as the secondary antibody. Hematoxylin was used as a counterstain. The immunostaining was performed on a Leica Biosystems BOND® RX instrument.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-LEF1 antibody [EPR2029Y] - BSA and Azide free (ab215999)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab137872**).

ab137872 staining LEF1 in paraffin embedded human melanoma tissue by Immunohistochemistry. Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins. Samples were incubated with primary antibody at 1/2000 dilution for 30 mins at room temperature. A ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) was used as the secondary antibody. Hematoxylin was used as a counterstain. The immunostaining was performed on a Leica Biosystems BOND® RX instrument.



Tissue Microarray (TMA) data for ab137872

Normal tissue samples				Malignant tissue samples			
Human cardiac muscle	x	Human placenta	x (immune cells ✓)	Clear cell carcinoma of human kidney	x	Human glioma	x (immune cells ✓)
Human cerebrum	x	Human skeletal muscle	x	Human bladder cancer	x (immune cells ✓)	Human hepatocellular carcinoma	x (immune cells ✓)
Human colon	x (immune cells ✓)	Human skin	x	Human breast carcinoma	✓	Human lung carcinoma	x (immune cells ✓)
Human endometrium	x	Human spleen	✓	Human cervical carcinoma	x (immune cells ✓)	Human ovarian carcinoma	x (immune cells ✓)
Human kidney	x	Human stomach	x (immune cells ✓)	Human colon carcinoma	✓	Human pancreatic carcinoma	x (immune cells ✓)
Human liver	x	Human testis	x	Human endometrial carcinoma	✓	Human prostatic hyperplasia	✓
Human lung	x	Human thyroid	x	Human gastric adenocarcinoma	x (immune cells ✓)	Human thyroid carcinoma	✓
Human mammary gland	x	Human tonsil	✓	Human non-Hodgkin's lymphoma	✓	Human thymoma	✓
Human pancreas	x			Human Hodgkin's lymphoma	✓	Human melanoma	✓

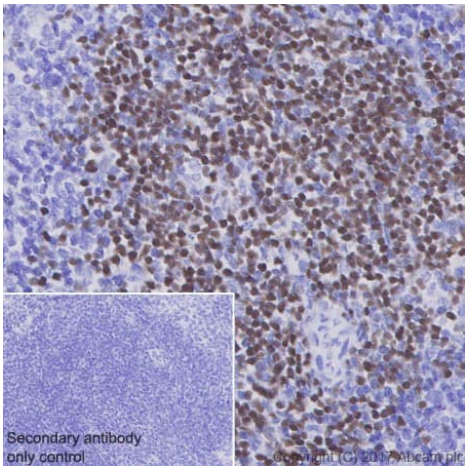
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-LEF1 antibody [EPR2029Y] - BSA and Azide free (ab215999)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab137872**).

Tissue Microarrays stained for Anti-LEF1 antibody [EPR2029Y] using **ab137872** in immunohistochemical analysis. This table provides a detailed overview of positive (tick mark) and negaive (cross mark) staining per sample type tested. The sections were incubated with **ab137872** for 30 mins at room temperature used at 1:2000 dilution (1.05 µg/ml) followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB secondary antibody (**ab209101**). Counterstain was Hematoxylin.

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins.

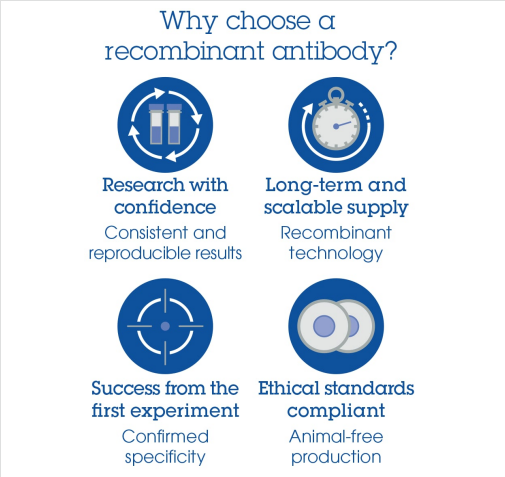
The immunostaining was performed on a Leica Biosystems BOND® RX instrument.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-LEF1 antibody [EPR2029Y] - BSA and Azide free (ab215999)

This IHC data was generated using the same anti-LEF1 antibody clone, EPR2029Y, in a different buffer formulation (cat# **ab137872**).

Ab137872 staining LEF1 in paraffin embedded Mouse spleen tissue by Immunohistochemistry. Antigen retrieval was by heat mediation using **ab93684** (Tris/EDTA buffer, Ph9). Samples were incubated with primary antibody at 1:2000 dilution. A ready to use Goat Anti-rabbit IgG H&L (HRP) was used as the secondary antibody. Hematoxylin was used as a counter stain. Nuclear staining on T cells in periarterial lymphatic sheath of mouse spleen (PMID: 21685909).



Anti-LEF1 antibody [EPR2029Y] - BSA and Azide free (ab215999)

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