abcam

Product datasheet

Anti-LEF1 antibody [EPR2029Y] ab137872

KO 評価済 リコンビナント RabMAb

3 Abreviews 54 References 画像数 20

製品の概要

製品名	Anti-LEF1 antibody [EPR2029Y]
製品の詳細	Rabbit monoclonal [EPR2029Y] to LEF1
由来種	Rabbit
アプリケーション	**適用あり:** IP, WB, IHC-P, ICC/IF, Flow Cyt (Intra)
種交差性	**交差種:** Mouse, Rat, Human
免疫原	Synthetic peptide within Human LEF1 aa 100-200. The exact sequence is proprietary. Database link: **Q9UJU2**
ポジティブ・コントロール	WB: Jurkat whole cell lysate (**ab7899**); Rat thymus tissue lysate; Human fetal lysate; His-tagged mouse LEF-1 recombinant protein (aa1-397). IHC-P: Human tonsil and thymus tissues; Mouse and rat spleen tissues. ICC/IF: Jurkat cells. Flow Cyt (intra): Jurkat cells.
特記事項	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**.

製品の特性

製品の状態	Liquid
保存方法	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
バッファー	Preservative: 0.01% Sodium azide Constituents: 40% Glycerol, 0.05% BSA, PBS
精製度	Protein A purified
ポリ/モノ	モノクローナル
クローン名	EPR2029Y

アイソタイプ IgG

アプリケーション

The Abpromise guarantee **Abpromise保証は、** 次のテスト済みアプリケーションにおけるab137872の使用に適用されます

アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
IP		Use at an assay dependent concentration.
WB		1/1000. Predicted molecular weight: 44 kDa. We don't recommend this antibody for mouse in Western Blot. In our hands an extra band was observed in mouse tissue lysates.
IHC-P	★★★★★ (2)	1/100 - 1/500. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**.
ICC/IF		1/500.
Flow Cyt (Intra)		Use at an assay dependent concentration.

ターゲット情報

機能 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.

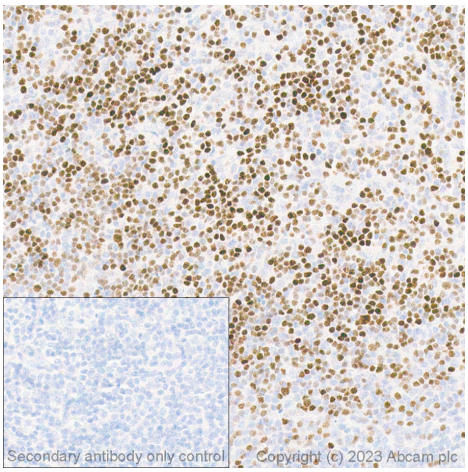
組織特異性 Detected in thymus. Not detected in normal colon, but highly expressed in colon cancer biopsies and colon cancer cell lines. Expressed in several pancreatic tumors and weakly expressed in normal pancreatic tissue. Isoforms 1 and 5 are detected in several pancreatic cell lines.

配列類似性 Belongs to the TCF/LEF family.
Contains 1 HMG box DNA-binding domain.

ドメイン Proline-rich and acidic regions are implicated in the activation functions of RNA polymerase II transcription factors.

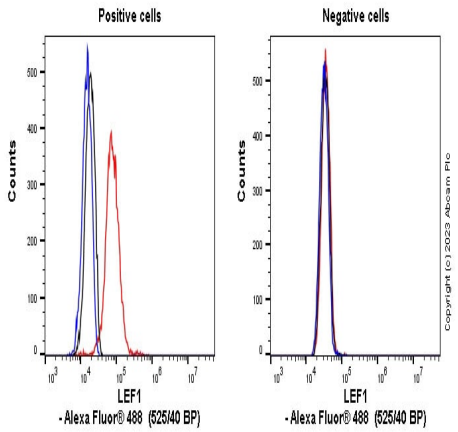
細胞内局在 Nucleus. Found in nuclear bodies upon PIASG binding.

画像



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-LEF1 antibody [EPR2029Y] (ab137872)

Immunohistochemical analysis of formalin fixed paraffin embedded human tonsil labelling LEF1 with ab137872 at a concentration of 0.5 µg/ml. The immunostaining was performed on a Ventana DISCOVERY ULTRA (Roche Tissue Diagnostics) instrument with an OptiView DAB IHC Detection Kit. Heat mediated antigen retrieval was conducted for 32 mins at 100°C with ULTRA cell conditioning solution (CC1, pH 8.5). ab137872 anti LEF1 antibody [EPR2029Y] was incubated at 37°C for 16 mins. Sections were counterstained with Hematoxylin II. Image inset shows absence of staining in secondary antibody only control.



Flow Cytometry (Intracellular) - Anti-LEF1 antibody [EPR2029Y] (ab137872)

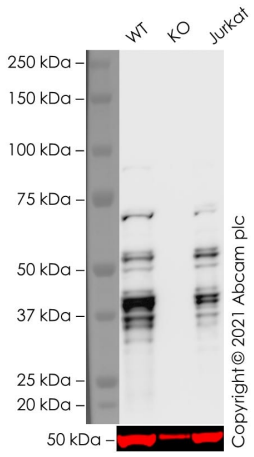
Flow cytometry overlay histogram showing left Jurkat positive cells and right negative HeLa stained with ab137872 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10% normal goat 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] (ab137872)

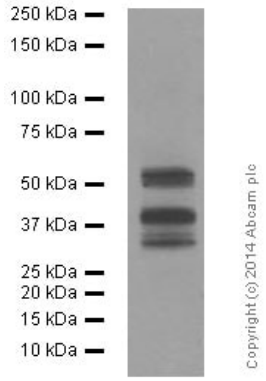
All lanes : Anti-LEF1 antibody [EPR2029Y] (ab137872) at 1/1000 dilution

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.



Western blot - Anti-LEF1 antibody [EPR2029Y] (ab137872)

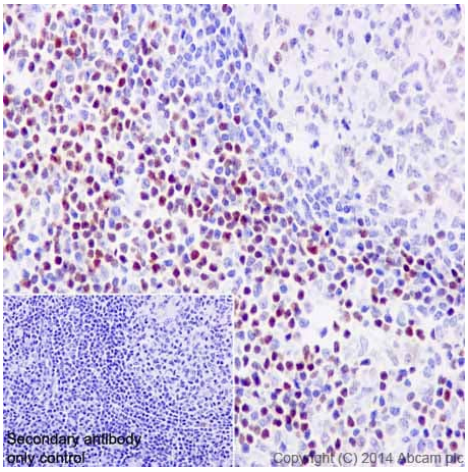
Anti-LEF1 antibody [EPR2029Y] (ab137872) at 1/1000 dilution (purified) + Human fetal thymus lysate at 10 µg

Secondary

HRP goat anti-rabbit (H+L) at 1/1000 dilution

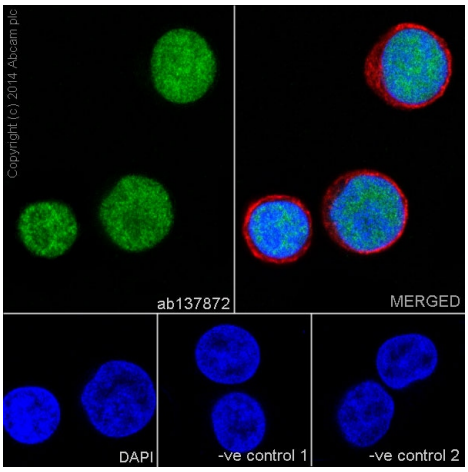
Predicted band size: 44 kDa

Blocking/Dilution buffer: 5% NFDM/TBST



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-LEF1 antibody [EPR2029Y] (ab137872)

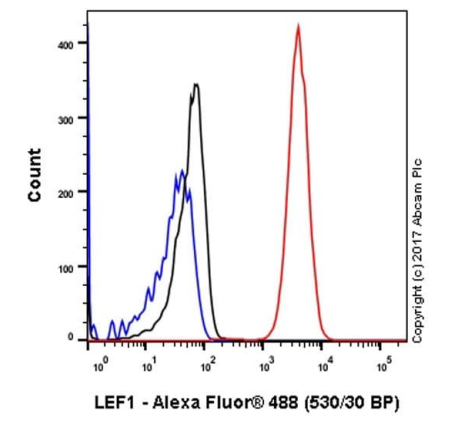
Immunohistochemical staining of paraffin embedded human tonsil with purified ab137872 at a working dilution of 1/500. The secondary antibody used is **ab97051**, an HRP-conjugated goat anti-rabbit IgG (H+L), at a dilution of 1/500. The sample is counter-stained with hematoxylin. Antigen retrieval was 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.



Immunocytochemistry/ Immunofluorescence - Anti-LEF1 antibody [EPR2029Y] (ab137872)

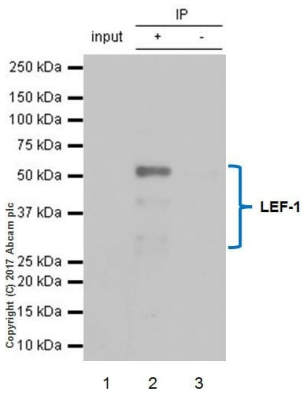
Immunofluorescence staining of Jurkat (Human T cell leukemia cell line from peripheral blood) cells with purified ab137872 at a working dilution of 1 in 500, counter-stained with DAPI. Tubulin was stained with mouse anti-tubulin at a dilution of 1/1000 (**ab7291**) and Alexa Fluor® 594 goat anti-mouse 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.



Flow Cytometry (Intracellular) - Anti-LEF1 antibody [EPR2029Y] (ab137872)

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.



Immunoprecipitation - Anti-LEF1 antibody [EPR2029Y] (ab137872)

Lane 1 (input): Jurkat (human T cell leukemia T lymphocyte) whole cell lysate, 10 µg

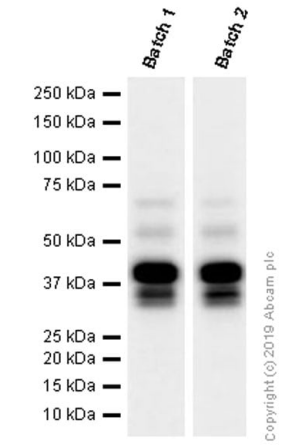
Lane 2 (+): Jurkat whole cell lysate

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. VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/10,000 dilution.

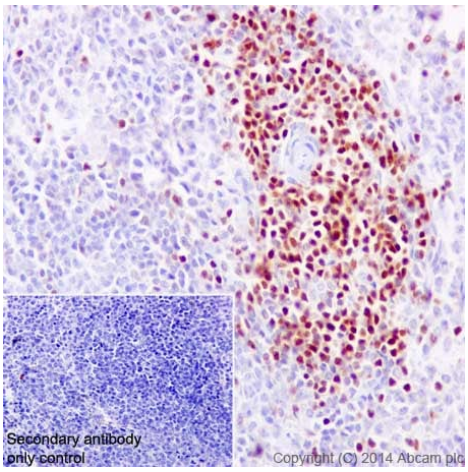
Blocking and diluting buffer: 5% NFDM/TBST

Exposure time: 3 minutes



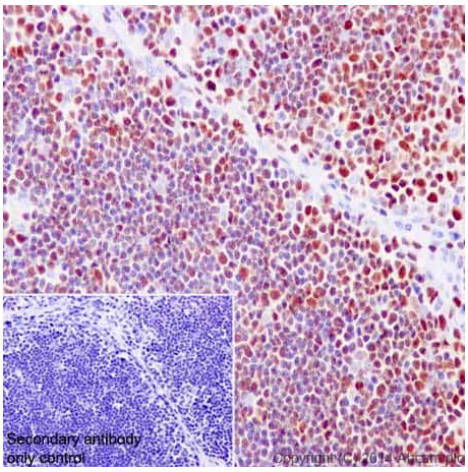
Western blot - Anti-LEF1 antibody [EPR2029Y] (ab137872)

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.



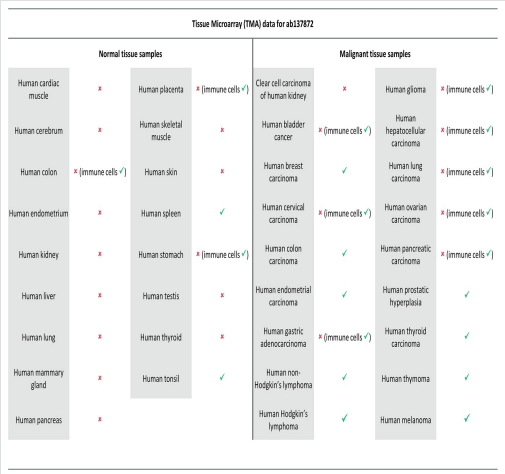
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-LEF1 antibody [EPR2029Y] (ab137872)

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.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-LEF1 antibody [EPR2029Y] (ab137872)

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.



Tissue Microarray (TMA) data for ab137872

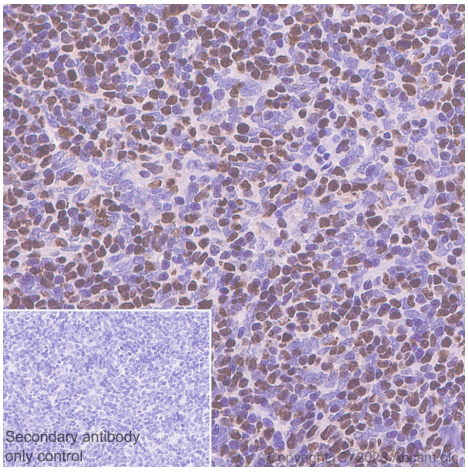
Normal tissue samples				Malignant tissue samples			
Human cardiac muscle	x	Human placenta	x (immune cells ✓)	Clear cell carcinoma of human kidney	x	Human glioma	x (immune cells ✓)
Human cerebrum	x	Human skeletal muscle	x	Human bladder cancer	x (immune cells ✓)	Human hepatocellular carcinoma	x (immune 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] (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.

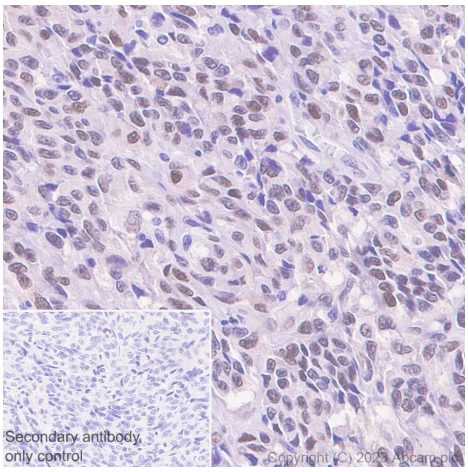
Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins.

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



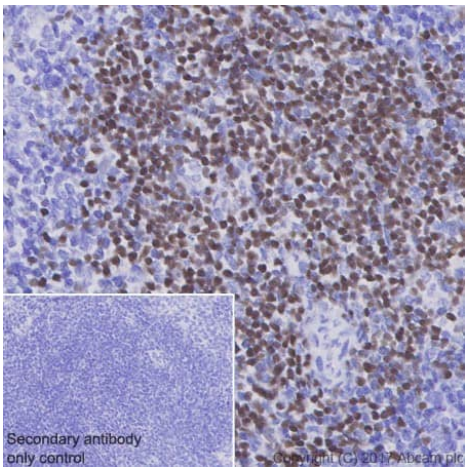
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-LEF1 antibody [EPR2029Y] (ab137872)

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.



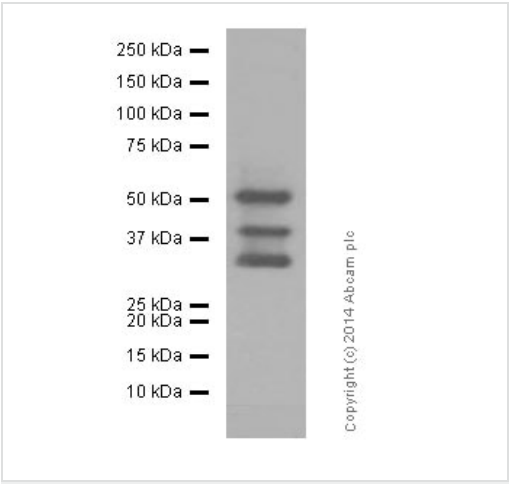
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-LEF1 antibody [EPR2029Y] (ab137872)

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.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-LEF1 antibody [EPR2029Y] (ab137872)

ab137872 staining LEF1 in paraffin embedded mouse spleen tissue by Immunohistochemistry. Antigen retrieval was performed by heat mediation using **ab93684** (Tris/EDTA buffer, ph 9). 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 counterstain. Nuclear staining on T cells in periarterial lymphatic sheath of mouse spleen is observed (PMID: 21685909).



Western blot - Anti-LEF1 antibody [EPR2029Y] (ab137872)

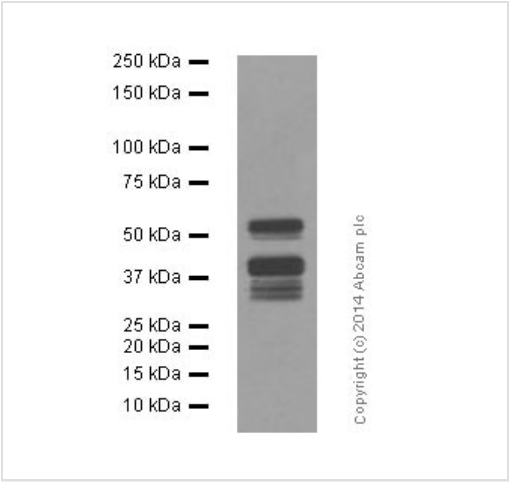
Anti-LEF1 antibody [EPR2029Y] (ab137872) at 1/2000 dilution (purified) + Rat thymus tissue lysate at 20 µg

Secondary

HRP goat anti-rabbit (H+L) at 1/1000 dilution

Predicted band size: 44 kDa

Blocking/Dilution buffer: 5% NFDM/TBST



Western blot - Anti-LEF1 antibody [EPR2029Y] (ab137872)

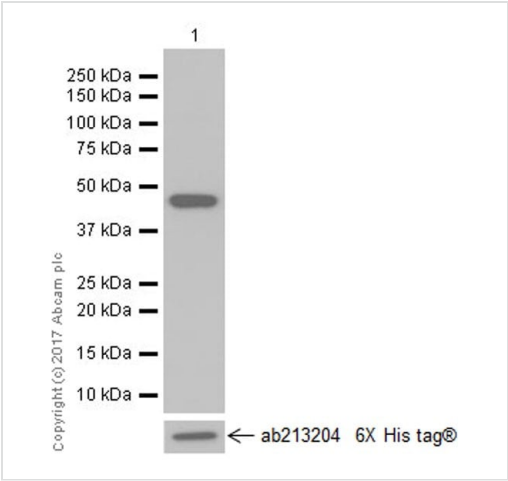
Anti-LEF1 antibody [EPR2029Y] (ab137872) at 1/10000 dilution (purified) + Jurkat (Human T cell leukemia cell line from peripheral blood) cell lysate at 10 µg

Secondary

HRP goat anti-rabbit (H+L) at 1/1000 dilution

Predicted band size: 44 kDa

Blocking/Dilution buffer: 5% NFDM/TBST



Western blot - Anti-LEF1 antibody [EPR2029Y] (ab137872)

Anti-LEF1 antibody [EPR2029Y] (ab137872) at 1/1000 dilution + His-tagged mouse LEF-1 recombinant protein (aa1-397) at 0.01 µg

Secondary

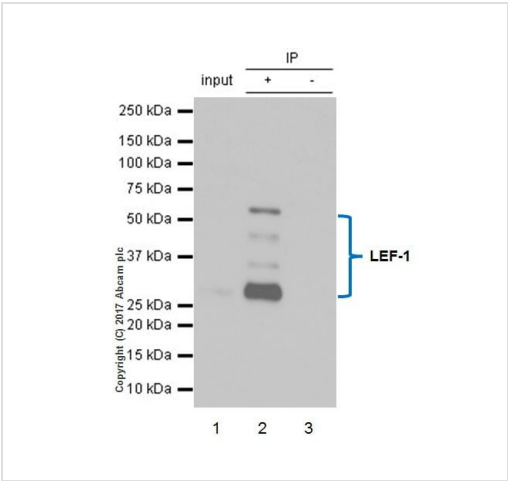
Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 44 kDa

Observed band size: 44 kDa

Exposure time: 1 second

Blocking and diluting buffer: 5% NFDM/TBST



Immunoprecipitation - Anti-LEF1 antibody [EPR2029Y] (ab137872)

Lane 1 (input): Rat thymus lysate, 10µg

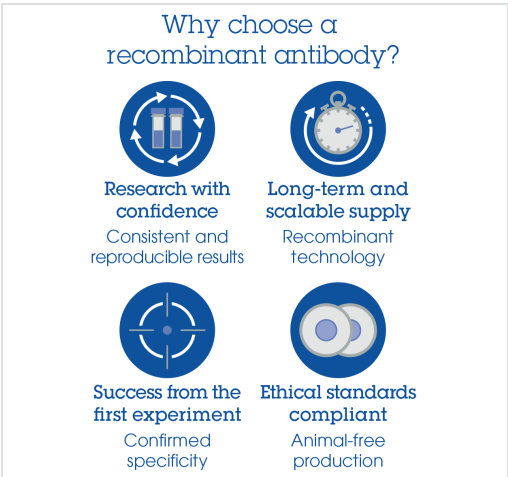
Lane 2 (+): Rat thymus lysate

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



Anti-LEF1 antibody [EPR2029Y] (ab137872)

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