

Product datasheet

Anti-PU.1/Spi1 antibody [EPR25123-110] ab302623

Recombinant RabMAb

13 Images

Overview

Product name	Anti-PU.1/Spi1 antibody [EPR25123-110]
Description	Rabbit monoclonal [EPR25123-110] to PU.1/Spi1
Host species	Rabbit
Tested applications	Suitable for: IP, ChIP, ChIC/CUT&RUN-seq, WB, ICC/IF, Flow Cyt (Intra), IHC-P
Species reactivity	Reacts with: Human Does not react with: Mouse, Rat
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: THP-1, U937, Daudi, HeLa whole cell lysates. IHC-P: Human colon and diffuse large B-cell lymphoma FFPE tissue sections. ICC/IF: THP-1 and U-937 cell lines. Flow Cyt (Intra): HeLa and U937 cells IP: THP-1 whole cell lysate. ChIP: U-937 cell line.
General notes	<p>This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or conjugation for your experiments, please contact orders@abcam.com.</p> <p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none">- High batch-to-batch consistency and reproducibility- Improved sensitivity and specificity- Long-term security of supply- Animal-free production <p>For more information see here.</p> <p>Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb[®] patents.</p>

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	EPR25123-110
Isotype	IgG

Applications

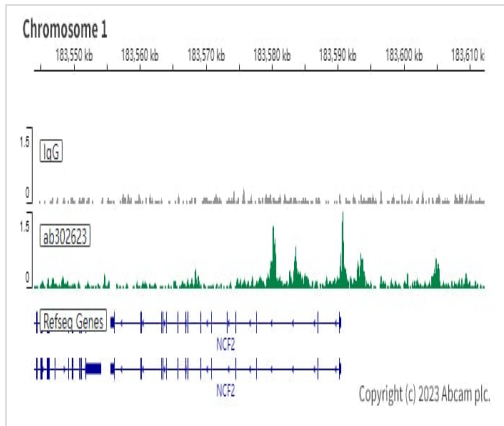
The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab302623 in the following tested applications. The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
IP		1/30.
ChIP		Use a concentration of 5 µg/ml.
ChIC/CUT&RUN-seq		Use at an assay dependent concentration.
WB		1/1000. Detects a band of approximately 31 kDa (predicted molecular weight: 31 kDa).
ICC/IF		1/250.
Flow Cyt (Intra)		1/500.
IHC-P		1/1000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Target

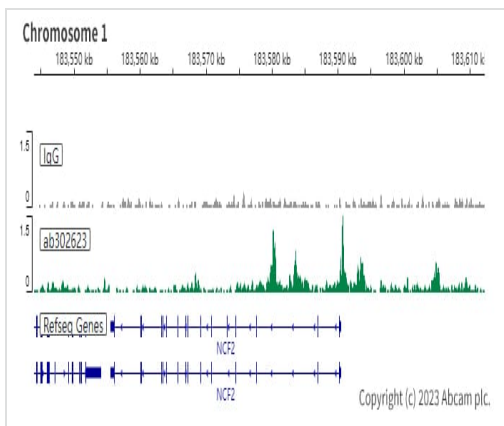
Function	Binds to the PU-box, a purine-rich DNA sequence (5'-GAGGAA-3') that can act as a lymphoid-specific enhancer. This protein is a transcriptional activator that may be specifically involved in the differentiation or activation of macrophages or B-cells. Also binds RNA and may modulate pre-mRNA splicing.
Sequence similarities	Belongs to the ETS family. Contains 1 ETS DNA-binding domain.
Cellular localization	Nucleus.

Images



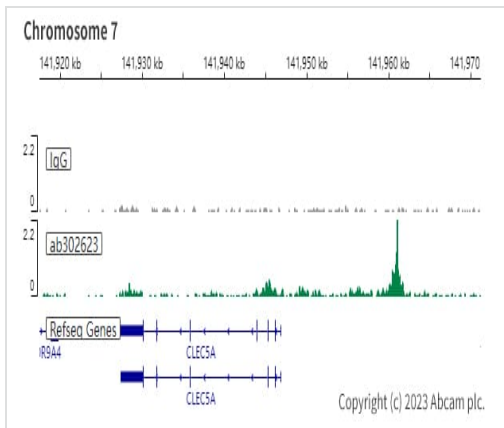
ChIC/CUT&RUN sequencing - Anti-PU.1/Spi1 antibody [EPR25123-110] (ab302623)

ChIC/CUT&RUN was performed using a pAG-MNase at a final concentration of 700 ng/mL, 2.5×10^5 THP-1 (Human monocytic leukemia monocyte) cells and 5 μ g of ab302623 [EPR25123-110]. The resulting DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 10 million reads. The negative IgG control **ab172730** is also shown. The University of Geneva owns patents relevant to ChIC (Chromatin Immuno-Cleavage) methods.



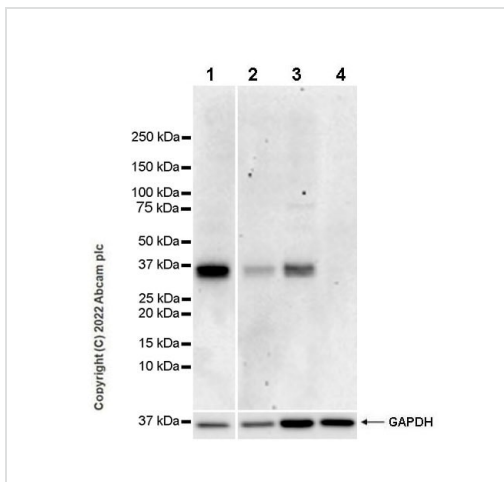
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Western blot - Anti-PU.1/Spi1 antibody [EPR25123-110] (AB302623)

All lanes : Anti-PU.1/Spi1 antibody [EPR25123-110] (ab302623) at 1/1000 dilution

Lane 1 : THP-1 (human monocytic leukemia monocyte), whole cell lysate

Lane 2 : U937 (human histiocytic lymphoma monocyte), whole cell lysate

Lane 3 : Daudi (human Burkitt's lymphoma lymphoblast), whole cell lysate

Lane 4 : HeLa (human cervix adenocarcinoma epithelial cell), whole cell lysate

Lysates/proteins at 20 μ g per lane.

Secondary

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

Predicted band size: 31 kDa

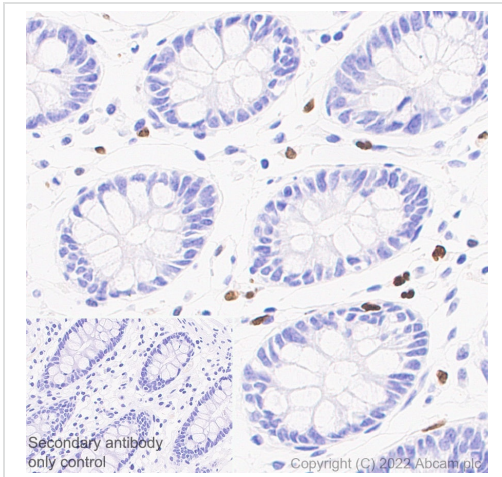
Observed band size: 31 kDa

Exposure time: 3 minutes

Blocking / Diluting buffer and concentration: 5% NFDM/TBST

Negative control: HeLa (PMID: 27010793)

This blot was developed using a high sensitivity ECL substrate.

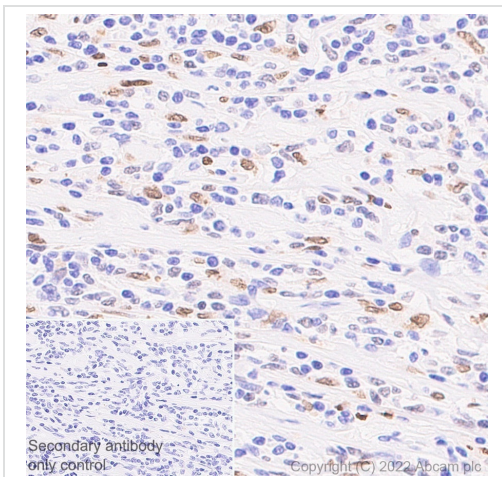


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PU.1/Spi1 antibody [EPR25123-110] (AB302623)

Immunohistochemical analysis of paraffin-embedded human colon tissue labeling PU.1/Spi1 with ab302623 at 1/1000 (0.467 µg/ml) followed by a ready to use Leica DS9800 (Bond™ Polymer Refine Detection kit). Positive staining in immune cells of human colon (PMID: 28681454). The section was incubated with ab302623 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Leica DS9800 (Bond™ Polymer Refine Detection kit).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins

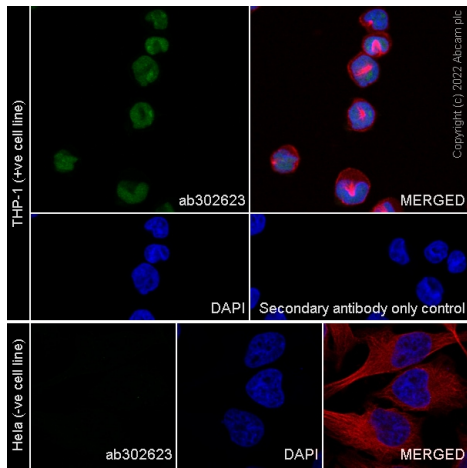


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PU.1/Spi1 antibody [EPR25123-110] (AB302623)

Immunohistochemical analysis of paraffin-embedded human diffuse large B-cell lymphoma tissue labeling PU.1/Spi1 with ab302623 at 1/1000 (0.467 µg/ml) followed by a ready to use Leica DS9800 (Bond™ Polymer Refine Detection kit). Positive staining in human diffuse large B-cell lymphoma (PMID:16648862). The section was incubated with ab302623 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Leica DS9800 (Bond™ Polymer Refine Detection kit).

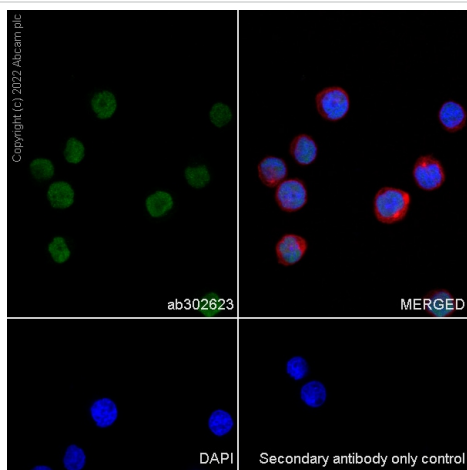
Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins



Immunocytochemistry/ Immunofluorescence - Anti-PU.1/Spi1 antibody [EPR25123-110] (AB302623)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized THP-1 (human monocytic leukemia monocyte) cells labeling PU.1/Spi1 with ab302623 at 1/250 (1.868 µg/ml) dilution, followed by **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 (2µg/ml) dilution (Green). Confocal image showing nuclear staining in THP-1 cell line. Negative control: HeLa (PMID: 27010793). **ab195889** Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 (2.5µg/ml) dilution (Red). The Nuclear counterstain was DAPI (Blue).

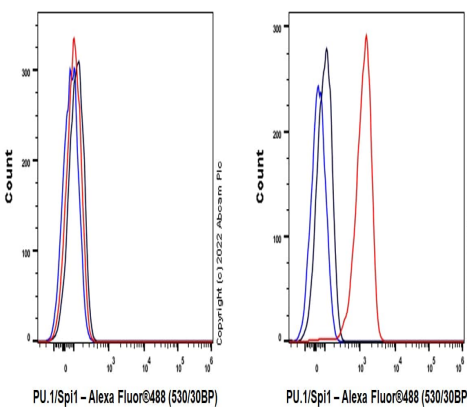
Secondary antibody only control: Secondary antibody is **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 (2µg/ml) dilution.



Immunocytochemistry/ Immunofluorescence - Anti-PU.1/Spi1 antibody [EPR25123-110] (AB302623)

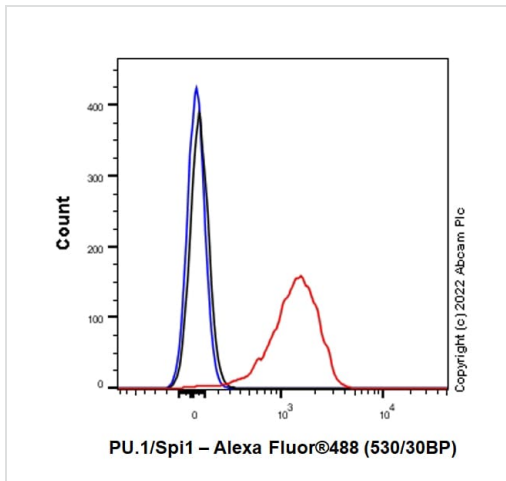
Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized U-937 (human histiocytic lymphoma monocyte) cells labeling PU.1/Spi1 with ab302623 at 1/250 (1.868 µg/ml) dilution, followed by **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 (2µg/ml) dilution (Green). Confocal image showing nuclear staining in U-937 cell line. **ab195889** Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 (2.5µg/ml) dilution (Red). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 (2µg/ml) dilution.



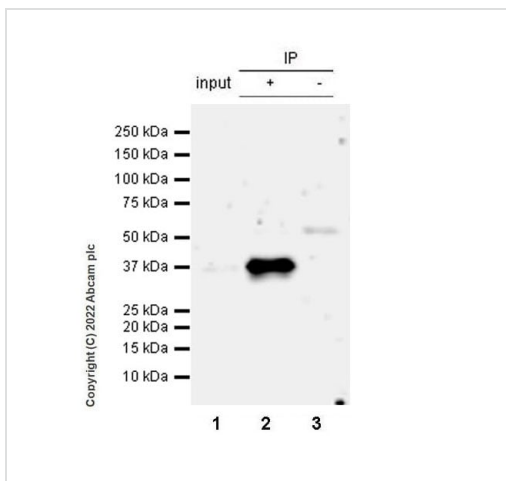
Flow Cytometry (Intracellular) - Anti-PU.1/Spi1 antibody [EPR25123-110] (ab302623)

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized HeLa (human cervix adenocarcinoma epithelial cell, Left) / U937 (human histiocytic lymphoma monocyte, Right) cells labeling PU.1/Spi1 with ab302623 at 1/500 dilution (0.1µg) (Red) compared with a Rabbit monoclonal IgG (**ab172730**) (Black) isotype control and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat Anti-Rabbit IgG (Alexa Fluor® 488, **ab150081**) at 1/2000 dilution was used as the secondary antibody. Negative control: HeLa (PMID: 27010793)



Flow Cytometry (Intracellular) - Anti-PU.1/Spi1 antibody [EPR25123-110] (ab302623)

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized THP-1 (human monocytic leukemia monocyte) cells labeling PU.1/Spi1 with ab302623 at 1/500 dilution (0.1 µg) (Red) compared with a Rabbit monoclonal IgG (**ab172730**) (Black) isotype control and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat Anti-Rabbit IgG (Alexa Fluor® 488, **ab150081**) at 1/2000 dilution was used as the secondary antibody.



Immunoprecipitation - Anti-PU.1/Spi1 antibody [EPR25123-110] (AB302623)

PU.1/Spi1 was immunoprecipitated from 0.35 mg THP-1 (human monocytic leukemia monocyte), whole cell lysate 10 µg with ab302623 at 1/30 dilution (2 µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab302623 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP) (**ab131366**) was used at 1/5000 dilution.

Lane 1: THP-1 (human monocytic leukemia monocyte), whole cell lysate 10 µg

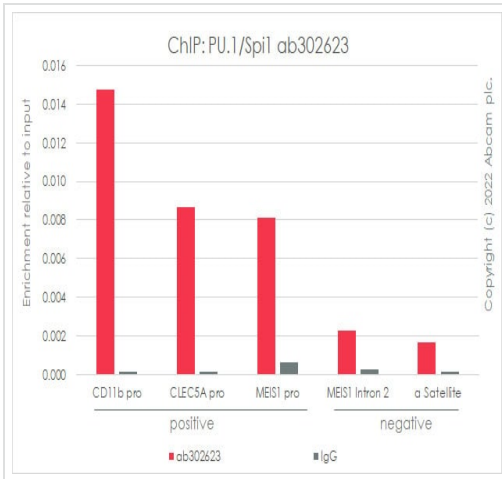
Lane 2: ab302623 IP in THP-1 whole cell lysate

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab302623 in THP-1 whole cell lysate

Blocking and dilution buffer and concentration: 5% NFD/MTBST.

Observed MW(KDa): 31 kDa

Exposure time: 3 minutes




ChIP - Anti-PU.1/Spi1 antibody [EPR25123-110]
(AB302623)


Chromatin was prepared from U-937 cells according to the Abcam Dual-X-ChIP protocol. Cells were fixed with 1.5 mM EGS for 30mins and then formaldehyde for 10min. The ChIP was performed with 25 µg of chromatin, 5 µg of ab302623 (red), or 5 µg of rabbit normal IgG **ab172730** (gray) and 25 µl of Protein A/G Dynabeads. The immunoprecipitated DNA was quantified by real time PCR (Sybr green approach).

Primers and probes are from paper PMID:21402070, 21094529, 26622774


Why choose a recombinant antibody?




Research with confidence
Consistent and reproducible results



Long-term and scalable supply
Recombinant technology



Success from the first experiment
Confirmed specificity



Ethical standards compliant
Animal-free production

Anti-PU.1/Spi1 antibody [EPR25123-110]
(AB302623)

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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