abcam

Product datasheet

Anti-PU.1/Spi1 antibody [EPR25123-110] (BSA and Azide free) ab302624

Recombinant RabMAb

10 Images

Overview

Product name	Anti-PU.1/Spi1 antibody [EPR25123-110] (BSA and Azide free)
Description	Rabbit monoclonal [EPR25123-110] to PU.1/Spi1 - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** IP, ChIP, Flow Cyt (Intra), IHC-P, WB, ICC/IF
Species reactivity	**Reacts with:** Human **Does not react with:** Mouse, Rat
Immunogen	Recombinant full length protein. 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	ab302624 is the carrier-free version of ab302623.

This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or conjugation for your experiments, please contact orders@abcam.com.

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.
Storage buffer	pH: 7.20 Constituent: 100% PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR25123-110
Isotype	IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab302624 in the following tested applications.

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

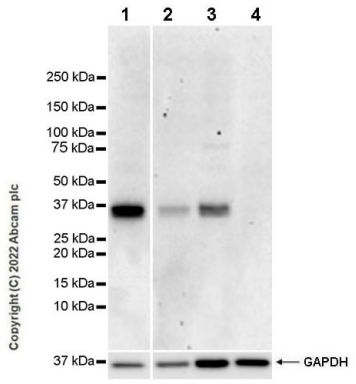
Application	Abreviews	Notes
IP		Use at an assay dependent concentration.
ChIP		Use at an assay dependent concentration.
Flow Cyt (Intra)		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB		Use at an assay dependent concentration. Detects a band of approximately 31 kDa (predicted molecular weight: 31 kDa).
ICC/IF		Use at an assay dependent concentration.

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



Western blot - Anti-PU.1/Spi1 antibody [EPR25123-110] (BSA and Azide free) (AB302624)

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 µg per lane.

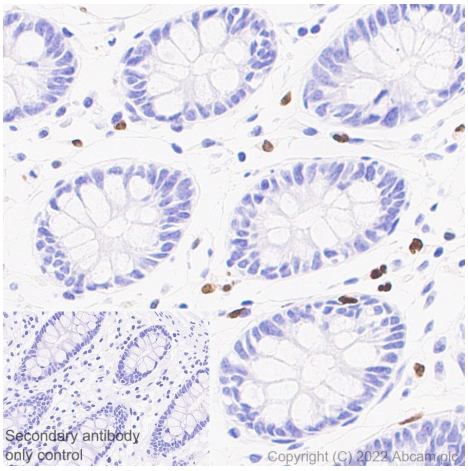
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Predicted band size: 31 kDa
Observed band size: 31 kDa

This data was developed using ab302623, the same antibody clone in a different buffer formulation.

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

Negative control: HeLa (PMID: 27010793)
This blot was developed using a high sensitivity ECL substrate.



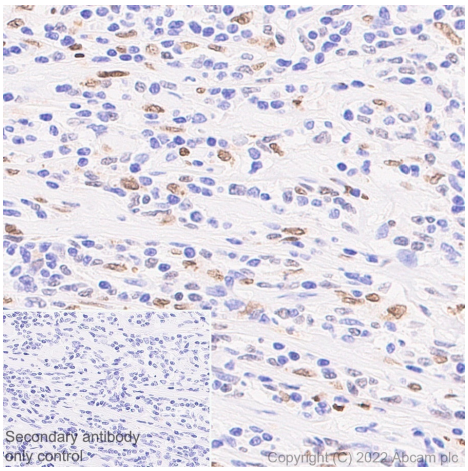
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PU.1/Spi1 antibody [EPR25123-110] (BSA and Azide free) (AB302624)

This data was developed using ab302623, the same antibody clone in a different buffer formulation.

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



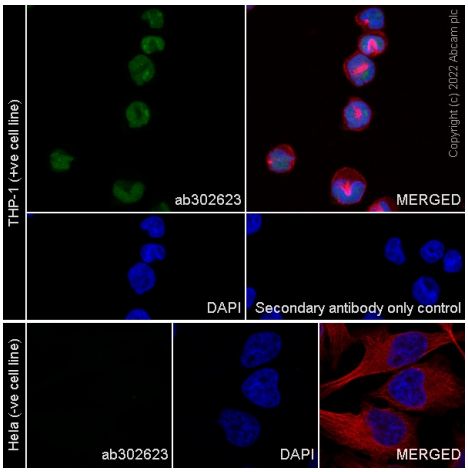
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PU.1/Spi1 antibody [EPR25123-110] (BSA and Azide free) (AB302624)

This data was developed using ab302623, the same antibody clone in a different buffer formulation.

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

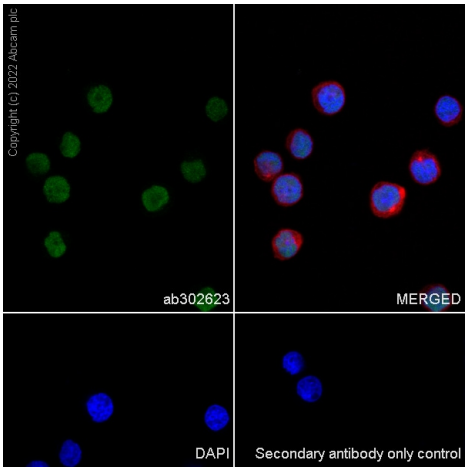


Immunocytochemistry/ Immunofluorescence - Anti-PU.1/Spi1 antibody [EPR25123-110] (BSA and Azide free) (AB302624)

This data was developed using ab302623, the same antibody clone in a different buffer formulation.

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.

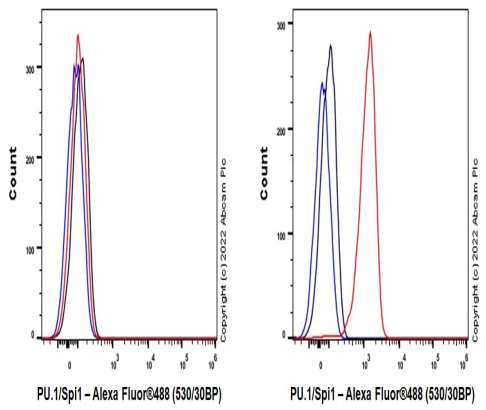


Immunocytochemistry/ Immunofluorescence - Anti-PU.1/Spi1 antibody [EPR25123-110] (BSA and Azide free) (AB302624)

This data was developed using ab302623, the same antibody clone in a different buffer formulation.

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).

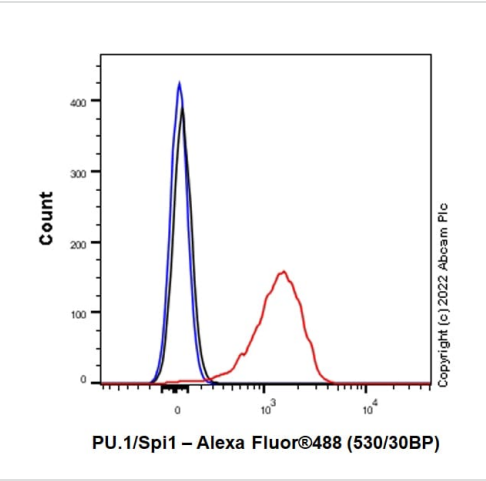
Secondary antibody only control: Secondary antibody is ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 (2µg/ml) dilution.



Flow Cytometry (Intracellular) - Anti-PU.1/Spi1 antibody [EPR25123-110] (BSA and Azide free) (ab302624)

This data was developed using ab302623, the same antibody clone in a different buffer formulation.

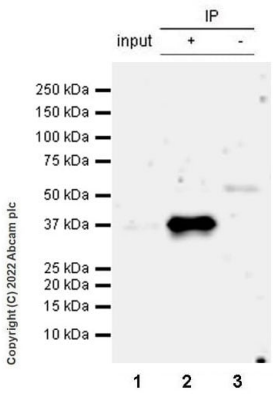
Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized HeLa (human cervix adenocarcinoma epithelial cell, Left) / U937 (human histiocytic lymphoma monocyte, Right) cells labeling PU.1/Spi1 with ab302623 at 1/500 dilution (0.1µg) (Red) compared with a Rabbit monoclonal IgG (ab172730) (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] (BSA and Azide free) (ab302624)

This data was developed using ab302623, the same antibody clone in a different buffer formulation.

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 (0.1µg) (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.



Immunoprecipitation - Anti-PU.1/Spi1 antibody [EPR25123-110] (BSA and Azide free) (AB302624)

This data was developed using ab302623, the same antibody clone in a different buffer formulation.

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 (2µg 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

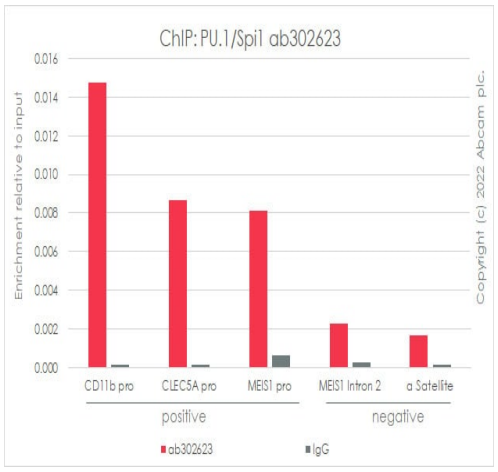
Lane 2: ab302623 IP in THP-1 whole cell lysate

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

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Observed MW(KDa): 31 kDa

Exposure time: 3 minutes

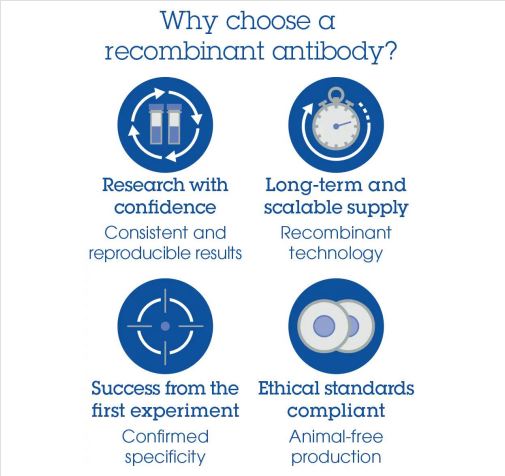


ChIP - Anti-PU.1/Spi1 antibody [EPR25123-110] (BSA and Azide free) (AB302624)

This data was developed using ab302623, the same antibody clone in a different buffer formulation.

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



Anti-PU.1/Spi1 antibody [EPR25123-110] (BSA and Azide free) (AB302624)

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