abcam

ab227835

Anti-PU.1/Spi1 antibody [EPR22624-20] - ChIP Grade

Recombinant | RabMAb | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EPR22624-20
Purification technique	Affinity purification Protein A
Concentration	0.516 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Tested

Species	Mouse
Dilution info	1/30
Notes	-

ChIP

Tested

Species	Mouse
Dilution info	5 µg for 25 µg chromatin
Notes	-

WB

Tested

Species	Mouse
Dilution info	1/1000
Notes	-

ICC/IF

Tested

Species	Mouse
Dilution info	1/500
Notes	-

Flow Cyt (Intra)

Tested

Species	Mouse
Dilution info	1/50
Notes	-

IHC-P

Tested

Species	Mouse
Dilution info	1/5000
Notes	

Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Target data

See full target information Spi1

Function	Pioneer transcription factor, which controls hematopoietic cell fate by decompacting stem cell heterochromatin and allowing other transcription factors to enter otherwise inaccessible genomic sites (PubMed:8079170). Once in open chromatin, can directly control gene expression by binding genetic regulatory elements and can also more broadly influence transcription by recruiting transcription factors, such as interferon regulatory factors (IRFs), to otherwise inaccessible genomic regions (By similarity). Transcriptionally activates genes important for myeloid and lymphoid lineages, such as CSF1R (By similarity). Transcriptional activation from certain promoters, possibly containing low affinity binding sites, is achieved cooperatively with other transcription factors. FCER1A transactivation is achieved in cooperation with GATA1 (By similarity). May be particularly important for the pro- to pre-B cell transition (PubMed:8079170). Binds (via the ETS domain) onto the purine-rich DNA core sequence 5'-GAGGAA-3', also known as the PU-box (PubMed:2180582). In vitro can bind RNA and interfere with pre-mRNA splicing (PubMed:8626664).

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	PU.1 also known as Spi1 is a transcription factor that belongs to the ETS family of transcription factors. It plays a major role in gene regulation and is an important player in hematopoietic cell differentiation. PU.1 has a molecular mass of approximately 31 kDa and is predominantly expressed in hematopoietic cells such as myeloid and B-lymphoid cells. It actively interacts with specific DNA sequences to regulate the expression of target genes.
Biological function summary	PU.1 influences the development and function of various blood cells including macrophages neutrophils and B-cells. It is essential for the regulation of genes involved in immune responses cell proliferation and survival. PU.1 functions as part of a larger transcriptional regulatory complex and often partners with other transcription factors and coactivators to exert its effects. This cooperation allows precise gene expression control in specific cell lineages.
Pathways	Expression of PU.1 is critical in the hematopoietic development pathway and the immune response pathway. It closely interacts with other transcription factors like GATA-1 and C/EBPα forming a network that affects hematopoietic lineage commitment. Within these pathways PU.1 constantly coordinates signals that influence progenitor cell fate and differentiation ensuring a balanced proportion of cell types within the blood.
Associated diseases and disorders	PU.1 has significant implications in leukemia and other hematological malignancies. Dysregulation or mutation of PU.1 can lead to improper hematopoietic cell development and contribute to acute myeloid leukemia (AML) and other blood disorders. Additionally its expression level is closely linked with immune system function associating PU.1 indirectly with autoimmune conditions. In these contexts the interaction with other proteins like AML1 and C/EBPγ can influence disease pathogenesis by modulating gene expression patterns critical for normal hematopoietic and immune function.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

We do not recommend this combination. It is not covered by our product promise.

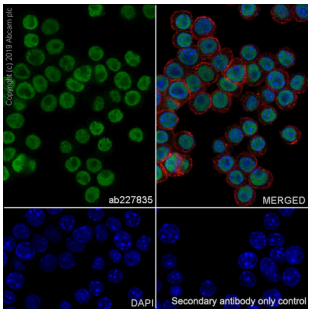
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

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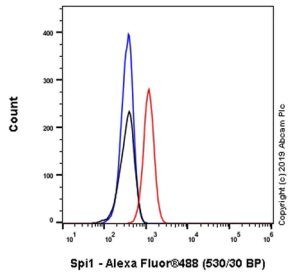
7 product images



Immunocytochemistry/ Immunofluorescence - Anti-PU.1/Spi1 antibody [EPR22624-20] - ChIP Grade (ab227835)

PU.1/Spi1 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-PU.1/Spi1 antibody

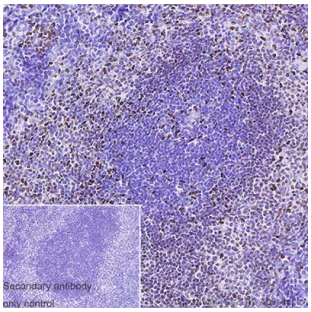
Immunofluorescent analysis of 4% parafoprmal;dehyde-fixed, 0.1% Triton X-100 permeabilized RAW 264.7 (Mouse Abelson murine leukemia virus-induced tumor macrophage) cells labeling PU.1/Spi1 with ab227835 at 1/500 dilution, followed by ab150077 AlexaFluor®488 Goat anti-Rabbit secondary at 1/1000 dilution (green). Confocal image showing nuclear staining in RAW 264.7 cell line. The nuclear counter stain is DAPI (blue). Tubulin is detected with ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) at 1/200 dilution (red). Secodary antibody only control: Used PBS instead of primary antibody, followed by ab150077 AlexaFluor®488 Goat anti-Rabbit secondary at 1/1000 dilution.



Flow Cytometry (Intracellular) - Anti-PU.1/Spi1 antibody [EPR22624-20] - ChIP Grade (ab227835)

PU.1/Spi1 Flow Cytometry (Intracellular) staining using rabbit Anti-PU.1/Spi1 antibody

Intracellular flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized RAW264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) cells labelling Spi1 with ab227835 at 1/50 dilution (Red) compared with a Rabbit monoclonal IgG (ab172730) isotype control (black)and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) at 1/2000 dilution was used as the secondary antibody.

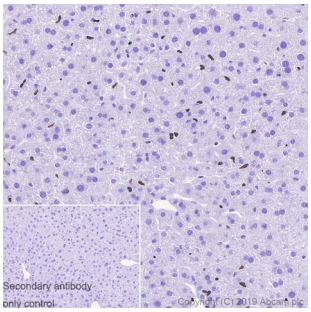


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PU.1/Spi1 antibody [EPR22624-20] - ChIP Grade (ab227835)

PU.1/Spi1 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-PU.1/Spi1 antibody

Immunohistochemical analysis of paraffin-embedded Mouse spleen tissue labeling Spi1 with ab227835 at 1/5000 dilution (0.103 ug/ml) followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Nuclear staining on mouse spleen. The section was incubated with ab227835 for 15 mins at RT. The immunostaining staining was performed on a Leica Biosystems BOND® RX instrument Counterstained with Hematoxylin.
Secondary antibody only control/ Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

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

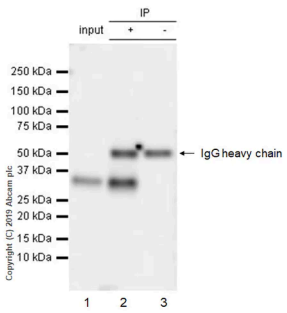


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PU.1/Spi1 antibody [EPR22624-20] - ChIP Grade (ab227835)

PU.1/Spi1 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-PU.1/Spi1 antibody

Immunohistochemical analysis of paraffin-embedded Mouse liver tissue labeling Spi1 with ab227835 at 1/5000 dilution (0.103 ug/ml) followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Nuclear staining in Kupffer cells of moue liver. The section was incubated with ab227835 for 15 mins at RT. The immunostaining staining was performed on a Leica Biosystems BOND® RX instrument Counterstained with Hematoxylin.
Secondary antibody only control/ Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

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



Immunoprecipitation - Anti-PU.1/Spi1 antibody [EPR22624-20] - ChIP Grade (ab227835)

Spi1 was immunoprecipitated from 0.35 mg J774A.1 (mouse reticulum cell sarcoma monocyte macrophage) whole cell lysate with ab227835 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab227835 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366) was used at 1/5000 dilution.
Lane 1: J774A.1 (mouse reticulum cell sarcoma monocyte macrophage) whole cell lysate 10ug

Lane 2: ab227835 IP in J774A.1 whole cell lysate

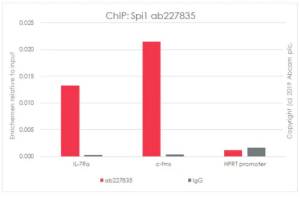
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab227835 in J774A.1 whole cell lysate

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

Exposure time: 30 seconds

All lanes:
Immunoprecipitation - Anti-PU.1/Spi1 antibody [EPR22624-20] - ChIP Grade (ab227835)

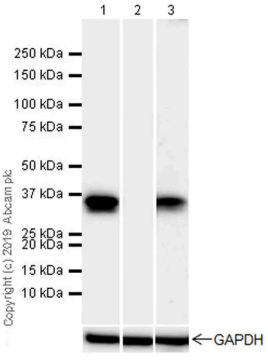
Predicted band size: 31 kDa



ChIP - Anti-PU.1/Spi1 antibody [EPR22624-20] - ChIP Grade (ab227835)

Chromatin was prepared from J774A.1 cells according to the Abcam Dual-X-ChIP protocol. Cells were fixed with 1.5 mM EGS for 30 mins and then formaldehyde for 10 min.
The ChIP was performed with 25 µg of chromatin, 5 µg of ab227835 (red), or 5 µg of rabbit normal IgG ab172730 (gray) and 20 µl of Protein A/G sepharose beads. The immunoprecipitated DNA was quantified by real time PCR (Taqman approach for active and inactive loci, Sybr green approach for heterochromatic loci).

Primers and probes are from paper PMID: 11869689



Western blot - Anti-PU.1/Spi1 antibody [EPR22624-20] - ChIP Grade (ab227835)

Blocking and dilution buffer: 5% NFDM/TBST .
Exposure times.

Lanes 1-2: 26 seconds; Lane 3: 3 minuters.

Negative control: NIH/3T3 cell line (PMID: 23509362, 16331260).

All lanes:
Western blot - Anti-PU.1/Spi1 antibody [EPR22624-20] - ChIP Grade (ab227835) at 1/1000 dilution

Lane 1:
RAW264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage), whole cell lysate at 20 µg

Lane 2:
NIH/3T3 (mouse embryonic fibroblast), whole cell lysate at 20 µg

Lane 3:
J774A.1 (mouse reticulum cell sarcoma monocyte macrophage), whole cell at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/50000 dilution

Predicted band size: 31 kDa

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.