abcam

Product datasheet

Anti-PU.1/Spi1 antibody [EPR22624-20] - ChIP Grade - BSA and Azide free ab264473

Recombinant RabMAb

7 Images

Overview

Product name	Anti-PU.1/Spi1 antibody [EPR22624-20] - ChIP Grade - BSA and Azide free
Description	Rabbit monoclonal [EPR22624-20] to PU.1/Spi1 - ChIP Grade – BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** ICC/IF, Flow Cyt (Intra), WB, IHC-P, IP, ChIP
Species reactivity	**Reacts with:** Mouse
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: RAW264.7, NIH/3T3 and J774A.1 lysates. IHC-P: Mouse spleen and Mouse liver tissues. ICC/IF: RAW 264.7 cells. Flow Cyt (intra): RAW264.7 cells. IP: J774A.1 cells.
General notes	ab264473 is the carrier-free version of **ab227835**. 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	EPR22624-20
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab264473 in the following tested applications.

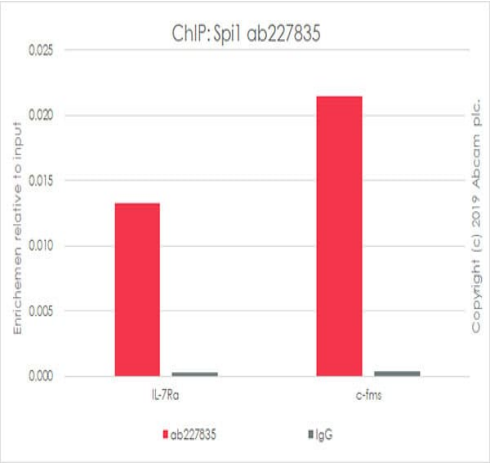
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ICC/IF		Use at an assay dependent concentration.
Flow Cyt (Intra)		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Predicted molecular weight: 31 kDa.
IHC-P		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.
ChIP		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

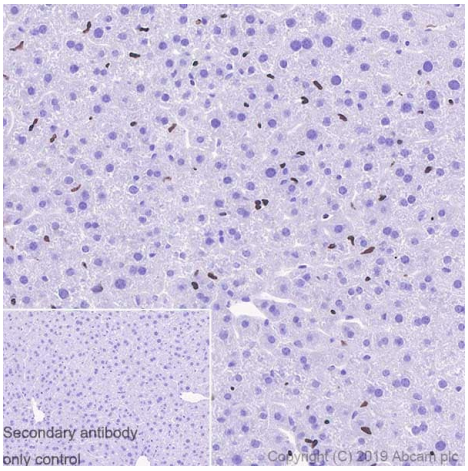


ChIP - Anti-PU.1/Spi1 antibody [EPR22624-20] - ChIP Grade - BSA and Azide free (ab264473)

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
***https://www.abcam.com/resources?keywords=X%20ChIP%20protocol**

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab227835**).



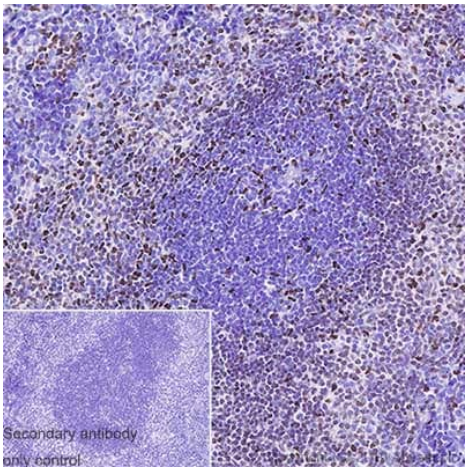
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PU.1/Spi1 antibody [EPR22624-20] - ChIP Grade - BSA and Azide free (ab264473)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab227835**).



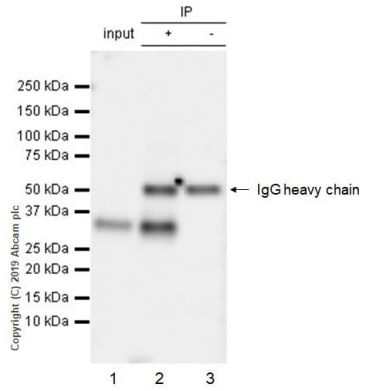
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PU.1/Spi1 antibody [EPR22624-20] - ChIP Grade - BSA and Azide free (ab264473)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab227835**).



Immunoprecipitation - Anti-PU.1/Spi1 antibody [EPR22624-20] - ChIP Grade - BSA and Azide free (ab264473)

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

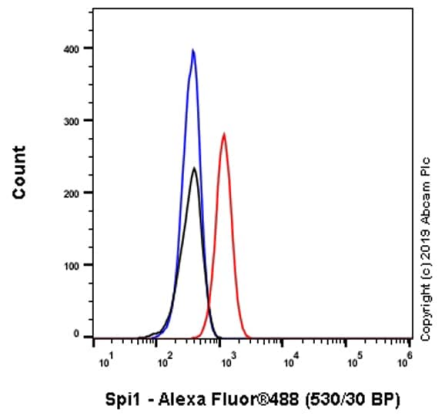
Lane 2: **ab227835** IP in J774A.1 whole cell lysate

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

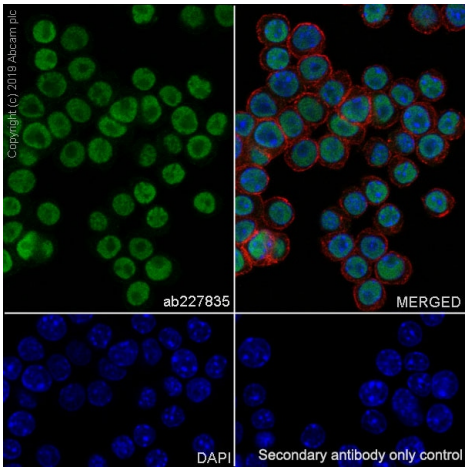
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab227835**).



Flow Cytometry (Intracellular) - Anti-PU.1/Spi1 antibody [EPR22624-20] - ChIP Grade - BSA and Azide free (ab264473)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab227835**).

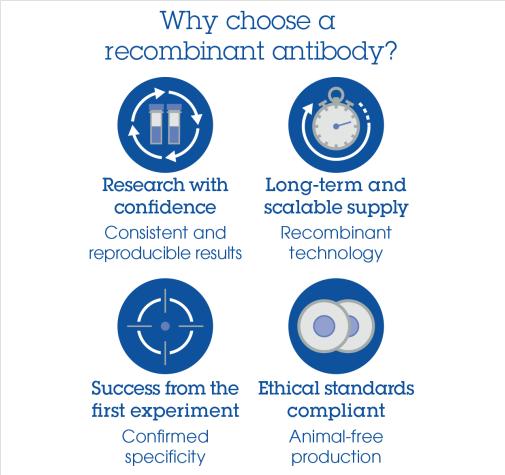


Immunocytochemistry/ Immunofluorescence - Anti-PU.1/Spi1 antibody [EPR22624-20] - ChIP Grade - BSA and Azide free (ab264473)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab227835**).



Anti-PU.1/Spi1 antibody [EPR22624-20] - ChIP Grade - BSA and Azide free (ab264473)

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