abcam

Product datasheet

Anti-PAX5 antibody [SP34] - ChIP Grade ab227635

Recombinant RabMAb

1 References 9 Images

Overview

Product name Anti-PAX5 antibody [SP34] - ChIP Grade

Description Rabbit monoclonal [SP34] to PAX5 - ChIP Grade

Host species Rabbit

Tested applications Suitable for: WB, IP, ChIP, IHC-P, Flow Cyt (Intra)

Species reactivity Reacts with: Rat, Human

Immunogen Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control Flow Cyt (Intra): Ramos cells. IHC-P: Human tonsil and B cell lymphoma and rat spleen tissues. IP:

Ramos whole cell lysates. WB: Ramos, Daudi, Jurkat and U-937 cell lysates.

General notes

This product is FOR RESEARCH USE ONLY. For commercial use, please contact

partnerships@abcam.com.

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: 7.60

Preservative: 0.1% Sodium azide Constituents: PBS, 1% BSA

Purity Protein A/G purified

Clonality Monoclonal

Clone number SP34
Isotype IgG

Applications

The Abpromise quarantee Our Abpromise guarantee covers the use of ab227635 in the following tested applications.

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

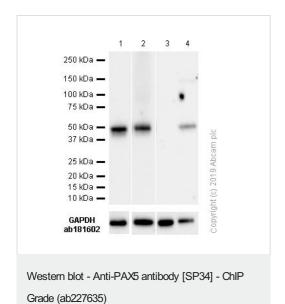
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Application	Abreviews	Notes
WB		Use a concentration of 0.172 µg/ml. Predicted molecular weight: 42 kDa.
IP		1/20.
ChIP		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration.
Flow Cyt (Intra)		1/20.

Target

Function	May play an important role in B-cell differentiation as well as neural development and spermatogenesis. Involved in the regulation of the CD19 gene, a B-lymphoid-specific target gene.	
Involvement in disease	A chromosomal aberration involving PAX5 is a cause of acute lymphoblastic leukemia. Translocation t(9;18)(p13;q11.2) with ZNF521. Translocation t(9;3)(p13;p14.1) with FOXP1. Translocation t(9;12)(p13;p13) with ETV6. Leukemia, acute lymphoblastic, 3	
Sequence similarities	Contains 1 paired domain.	
Developmental stage	Expressed at early B-cell differentiation, in the developing CNS and in adult testis.	
Post-translational modifications	O-glycosylated.	
Cellular localization	Nucleus.	

Images



All lanes : Anti-PAX5 antibody [SP34] - ChIP Grade (ab227635) at $0.172 \ \mu g/ml$

Lane 1 : Ramos (Human Burkitt's lymphoma B lymphocyte) cell lysate

Lane 2: Daudi (Human Burkitt's lymphoma lymphoblast) cell lysate
 Lane 3: Jurkat (Human T cell leukemia T lymphocyte) cell lysate
 Lane 4: U-937 (Human histiocytic lymphoma monocyte) cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

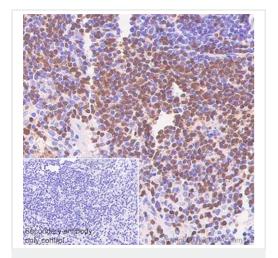
Lane 1 : Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 0.05 μ g Lanes 2-4 : Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 0.05 μ g/ml

Predicted band size: 42 kDa

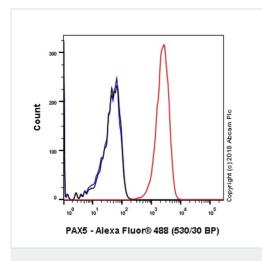
Blocking/Diluting buffer and concentration: 5% NFDM /TBST

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Rat spleen tissue sections labeling PAX5 with ab227635 at 1/50 dilution. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 6.0, epitope retrieval solution 1) for 10mins. Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) was used as the secondary antibody. Hematoxylin was used as a counterstain. Nuclear staining on the rat spleen, performed on a Leica Biosystems BOND™ RX instrument. The section was incubated with ab228136 for 30 mins at room

temperature.

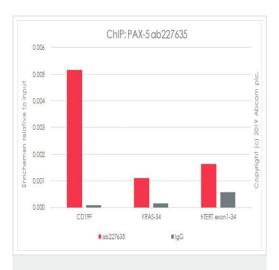


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PAX5 antibody [SP34] -ChIP Grade (ab227635)



Flow Cytometry (Intracellular) - Anti-PAX5 antibody [SP34] - ChIP Grade (ab227635)

Flow Cytometry analysis of Ramos(Human Burkitt's lymphoma B lymphocyte) cells labeling PAX5 with purified ab227635 at 1:20 dilution (8.6 µg/ml) Red. Cells were fixed with 4% paraformaldehyde . A Goat anti rabbit lgG (Alexa Fluor® 488, ab150081) secondary antibody was used at 1:2000 dilution. Isotype control - Rabbit monoclonal IgG (ab172730) / Black. Unlabelled control - Unlabelled cells / Blue.



ChIP - Anti-PAX5 antibody [SP34] - ChIP Grade (ab227635)

Chromatin was prepared from Ramos 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 μ g of chromatin, 5 μ g of ab227635 (red), or 5 μ g of rabbit normal lgG <u>ab172730</u> (gray) and 20 μ l 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 commercial primers from Paper (PMID: 19806612)

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



Immunoprecipitation - Anti-PAX5 antibody [SP34] - ChIP Grade (ab227635)

ab227635 (purified) at 1/20 dilution immunoprecipitating PAX5 in Ramos whole cell lysate.

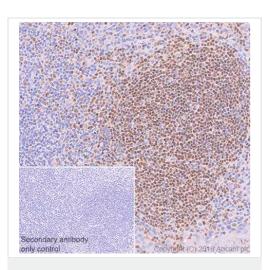
Lane 1 (input): Ramos(Human Burkitt's lymphoma B lymphocyte) whole cell lysate 10µg

Lane 2 (+): ab227635 & Ramos whole cell lysate

Lane 3 (-): Rabbit monoclonal IgG (ab172730) instead of ab227635 in Ramos whole cell lysate

For western blotting, $\underline{ab227648}$ (purified at 1/500 dilution (0.344 µg/ml) and veriBlot for IP secondary antibody (HRP) ($\underline{ab131366}$) at 1/1000 dilution were used.

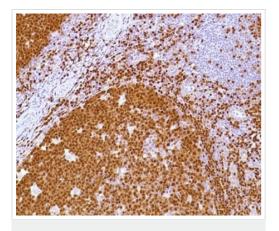
Blocking and diluting buffer: 5% NFDM /TBST.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PAX5 antibody [SP34] - ChIP Grade (ab227635)

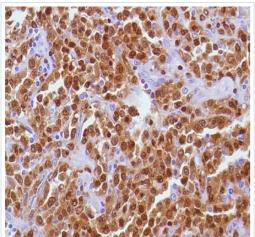
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human tonsil tissue sections labeling PAX5 with ab227635 at 1/50 dilution. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 6.0, epitope retrieval solution 1) for 10mins. Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) was used as the secondary antibody. Hematoxylin was used as a counterstain. Nuclear staining on the human tonsil, performed on a Leica Biosystems BOND™ RX instrument.

The section was incubated with <u>ab228136</u> for 30 mins at room temperature.



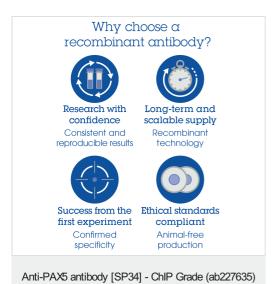
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PAX5 antibody [SP34] - ChIP Grade (ab227635)

Immunohistochemical analysis of Human tonsil tissue labeling PAX5 using ab227635.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PAX5 antibody [SP34] -ChIP Grade (ab227635)

Immunohistochemical analysis of Human B cell lymphoma tissue staining PAX5 using ab227635.



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