

Product datasheet

Anti-PAX5 antibody [EPR3730(2)] ab109443

Recombinant RabMAb

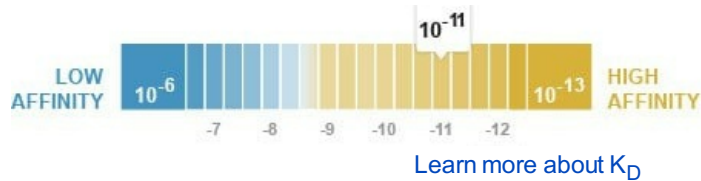
★★★★★ 3 Abreviews 17 References 20 Images

Overview

Product name	Anti-PAX5 antibody [EPR3730(2)]
Description	Rabbit monoclonal [EPR3730(2)] to PAX5
Host species	Rabbit
Tested applications	Suitable for: Flow Cyt (Intra), ICC/IF, IP, ChIP, WB, IHC-P
Species reactivity	Reacts with: Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Ramos and Daudi cell lysates and human tonsil and mouse spleen tissue lysates. IHC-P: Human tonsil, hodgkin's lymphoma, spleen and diffuse B-cell lymphoma tissues. ICC/IF: Ramos cells. Flow Cyt (intra): Ramos cells. IP: Mouse spleen tissue lysate and Ramos cells. ChIP: Ramos cells
General notes	<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> <p>We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.</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. Stable for 12 months at -20°C.
Dissociation constant (K_D)	K _D = 4.45 x 10 ⁻¹¹ M



Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 40% Glycerol, 59% PBS, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR3730(2)
Isotype	IgG

Applications

The Abpromise guarantee Our [Abpromise guarantee](#) covers the use of ab109443 in the following tested applications. The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

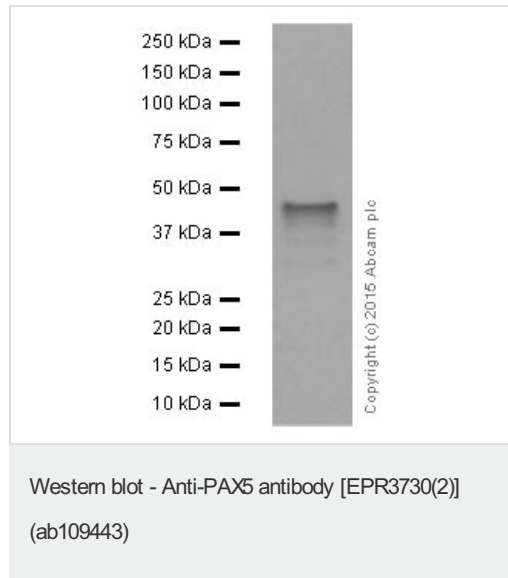
Application	Abreviews	Notes
Flow Cyt (Intra)		1/30. For unpurified use at 1/10 - 1/100. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ICC/IF	★★★★★ (1)	1/250. For unpurified use at 1/1000.
IP		1/20.
ChIP		Use at an assay dependent concentration.
WB		1/5000. Predicted molecular weight: 42 kDa. For unpurified use at 1/10000 - 1/50000.
IHC-P	★★★★★ (2)	1/5000. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See IHC antigen retrieval protocols . For unpurified use at 1/250 - 1/500.

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



Anti-PAX5 antibody [EPR3730(2)] (ab109443) at 1/5000 dilution (purified) + Mouse spleen tissue lysate at 20 μ g

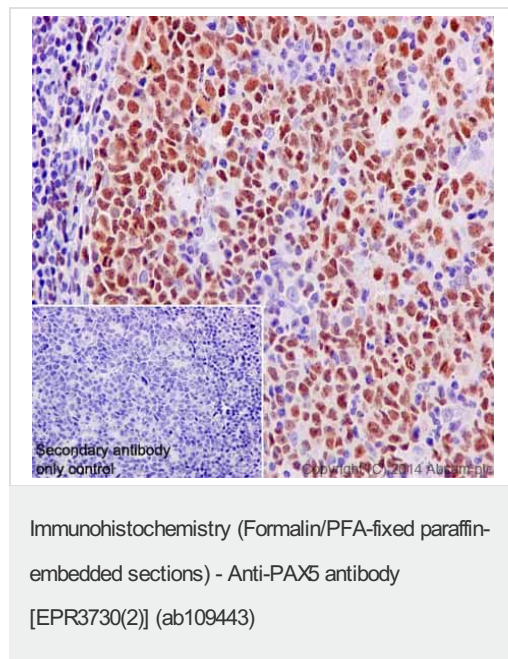
Secondary

Peroxidase-conjugated goat anti-rabbit IgG, (H+L) at 1/1000 dilution

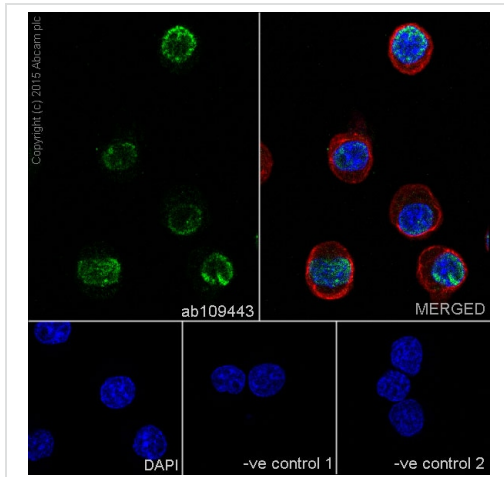
Predicted band size: 42 kDa

Observed band size: 45 kDa

Blocking and dilution buffer: 5% NFDm/TBST.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human tonsil tissue labelling PAX5 with purified ab109443 at 1/1000. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. [ab97051](#), a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.

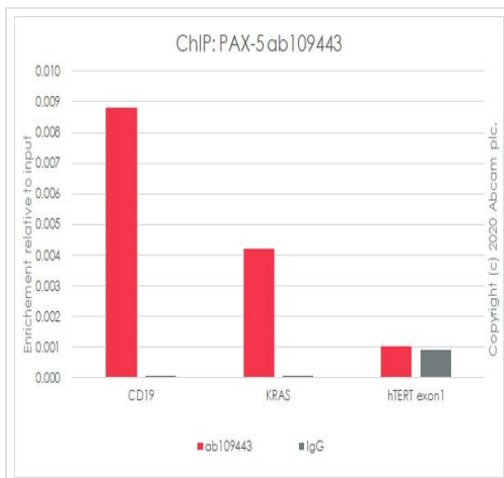


Immunocytochemistry/ Immunofluorescence - Anti-PAX5 antibody [EPR3730(2)] (ab109443)

Immunocytochemistry/Immunofluorescence analysis of Ramos cells labelling PAX5 with purified ab109443 at 1/250. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. **ab150077**, an Alexa Fluor[®] 488-conjugated goat anti-rabbit IgG (1/500) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain. **ab7291**, a mouse anti-tubulin (1/1000) and **ab150120**, an Alexa Fluor[®] 594-conjugated goat anti-mouse IgG (1/500) were also used.

Control 1: primary antibody (1/250) and secondary antibody, **ab150120**, an Alexa Fluor[®] 594-conjugated goat anti-mouse IgG (1/500).

Control 2: **ab7291** (1/1000) and secondary antibody, **ab150077**, an Alexa Fluor[®] 488-conjugated goat anti-rabbit IgG (1/500).



ChIP - Anti-PAX5 antibody [EPR3730(2)] (ab109443)

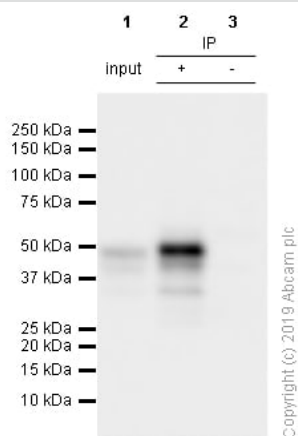
Chromatin was prepared from Ramos cells according to the Abcam Dual-X-ChIP protocol*. Cells were fixed with 1.5 mM EGS for 30mins and then formaldehyde for 10min.

The ChIP was performed with 25 µg of chromatin, 5 µg of abzzz (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:19806612

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

keywords=X%20ChIP%20protocol



Immunoprecipitation - Anti-PAX5 antibody
[EPR3730(2)] (ab109443)

ab109443 (purified) at 1/20 dilution immunoprecipitating PAX5 in mouse spleen lysate 10 µg.

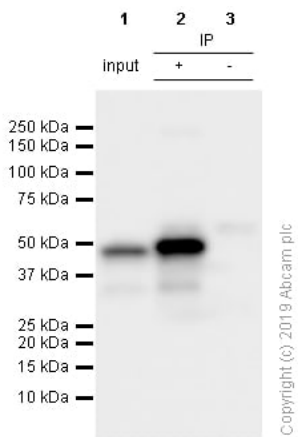
Lane 1 (input): mouse spleen lysate 10 µg

Lane 2 (+): ab109443 & mouse spleen lysate

Lane 3 (-): Rabbit monoclonal IgG ([ab172730](#)) instead of ab109443 in mouse spleen lysate

For western blotting, ab109443 at 1/500 dilution (0.268 µg/mL) and veriBlot for IP secondary antibody (HRP) ([ab131366](#)) at 1/1000 dilution was used.

Blocking and diluting buffer: 5% NFDM /TBST.



Immunoprecipitation - Anti-PAX5 antibody
[EPR3730(2)] (ab109443)

ab109443 (purified) at 1/20 dilution immunoprecipitating PAX5 in Ramos (Human Burkitt's lymphoma B lymphocyte) whole cell lysate 10 µg.

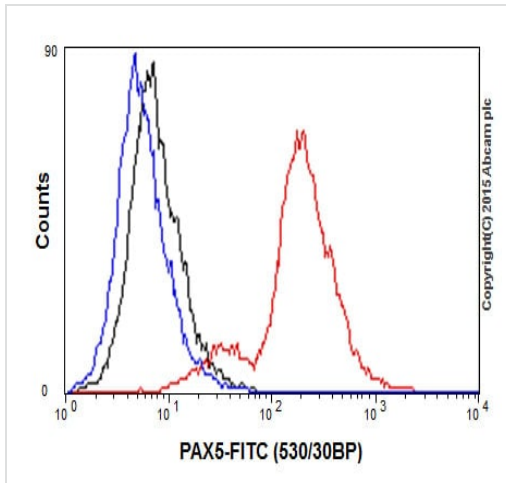
Lane 1 (input): Ramos (Human Burkitt's lymphoma B lymphocyte) whole cell lysate 10 µg

Lane 2 (+): ab109443 & Ramos whole cell lysate

Lane 3 (-): Rabbit monoclonal IgG ([ab172730](#)) instead of ab109443 in Ramos whole cell lysate

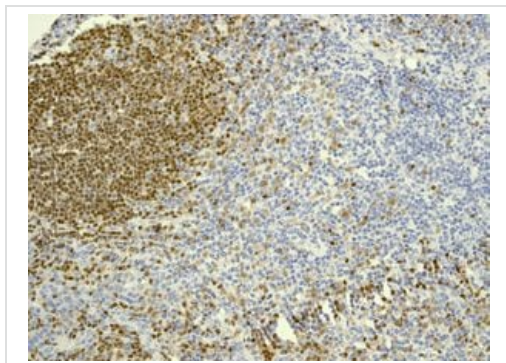
For western blotting, ab109443 at 1/500 dilution (0.268 µg/mL) and veriBlot for IP secondary antibody (HRP) ([ab131366](#)) at 1/1000 dilution was used.

Blocking and diluting buffer: 5% NFDM /TBST.



Flow Cytometry (Intracellular) - Anti-PAX5 antibody
[EPR3730(2)] (ab109443)

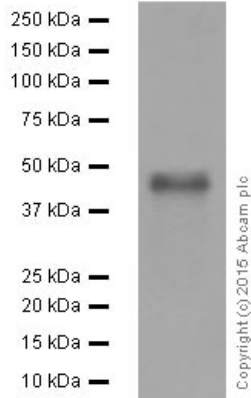
Intracellular Flow Cytometry analysis of Ramos cells labelling PAX5 with purified ab109443 at 1/30 (red). Cells were fixed with 80% methanol. A FITC-conjugated goat anti-rabbit IgG (1/150) was used as the secondary antibody. Black - Isotype control, rabbit monoclonal IgG. Blue - Unlabelled control, cells without incubation with primary and secondary antibodies.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PAX5 antibody
[EPR3730(2)] (ab109443)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human tonsil tissue labelling PAX5 with unpurified ab109443.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Western blot - Anti-PAX5 antibody [EPR3730(2)] (ab109443)

Anti-PAX5 antibody [EPR3730(2)] (ab109443) at 1/5000 dilution (purified) + Human tonsil tissue lysate at 20 µg

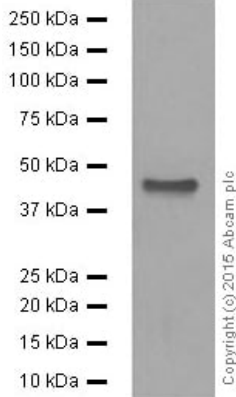
Secondary

Peroxidase-conjugated goat anti-rabbit IgG, (H+L) at 1/5000 dilution

Predicted band size: 42 kDa

Observed band size: 45 kDa

Blocking and dilution buffer: 5% NFDm/TBST.



Western blot - Anti-PAX5 antibody [EPR3730(2)] (ab109443)

Anti-PAX5 antibody [EPR3730(2)] (ab109443) at 1/20000 dilution (purified) + Ramos cell lysate at 20 µg

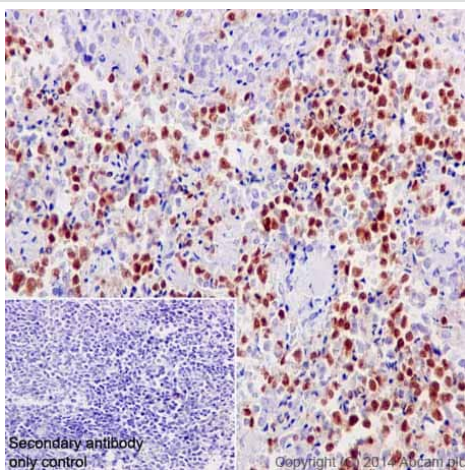
Secondary

Peroxidase-conjugated goat anti-rabbit IgG, (H+L) at 1/1000 dilution

Predicted band size: 42 kDa

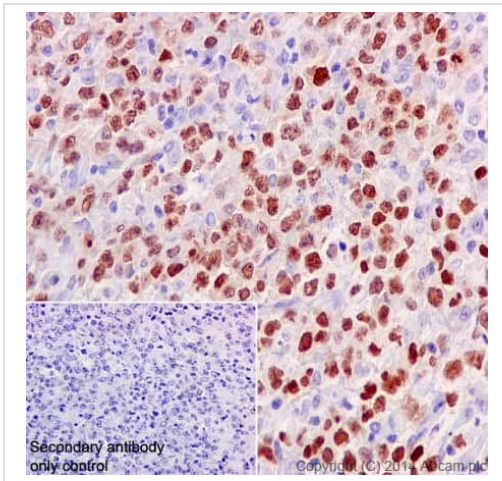
Observed band size: 45 kDa

Blocking and dilution buffer: 5% NFDm/TBST.



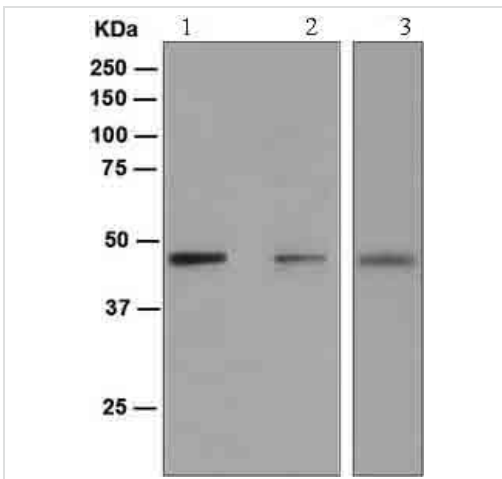
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PAX5 antibody [EPR3730(2)] (ab109443)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of rat spleen tissue labelling PAX5 with purified ab109443 at 1/1000. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. [ab97051](#), a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human B-cell lymphoma tissue labelling PAX5 with purified ab109443 at 1/1000. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. [ab97051](#), a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PAX5 antibody [EPR3730(2)] (ab109443)



Western blot - Anti-PAX5 antibody [EPR3730(2)] (ab109443)

All lanes : Anti-PAX5 antibody [EPR3730(2)] (ab109443) at 1/10000 dilution (unpurified)

Lane 1 : Ramos cell lysate

Lane 2 : Human tonsil tissue lysate

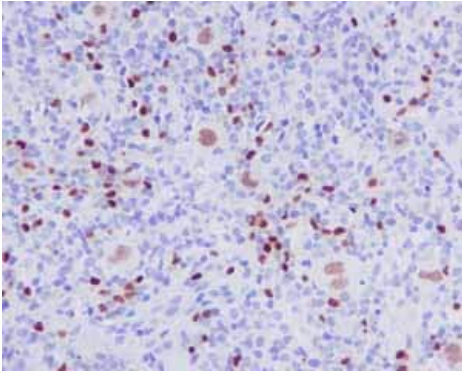
Lane 3 : Daudi cell lysates

Lysates/proteins at 10 µg per lane.

Secondary

All lanes : Standard HRP labelled goat anti-rabbit IgG at 1/2000 dilution

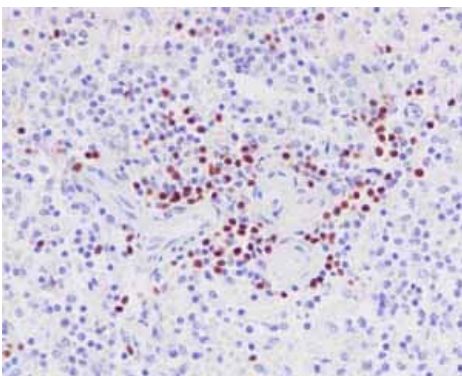
Predicted band size: 42 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PAX5 antibody
[EPR3730(2)] (ab109443)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human Hodgkin's lymphoma tissue labelling PAX5 with unpurified ab109443.

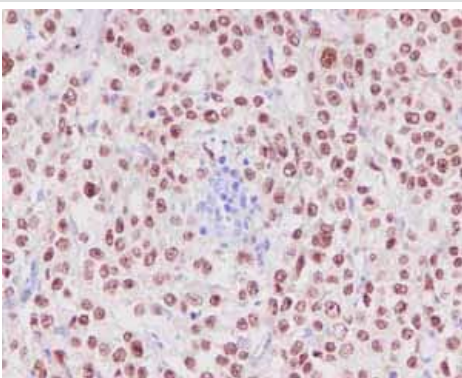
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PAX5 antibody
[EPR3730(2)] (ab109443)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human normal spleen tissue labelling PAX5 with unpurified ab109443.

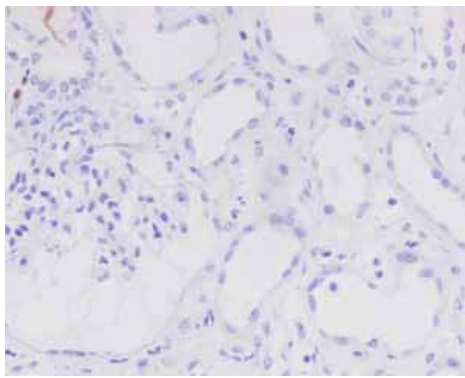
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PAX5 antibody
[EPR3730(2)] (ab109443)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human diffuse B-cell lymphoma tissue labelling PAX5 with unpurified ab109443.

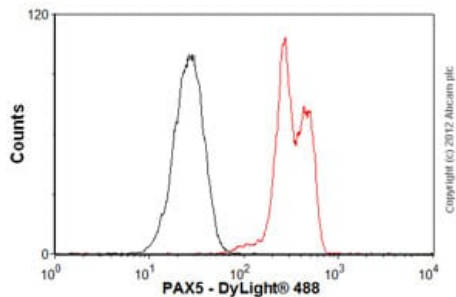
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PAX5 antibody [EPR3730(2)] (ab109443)

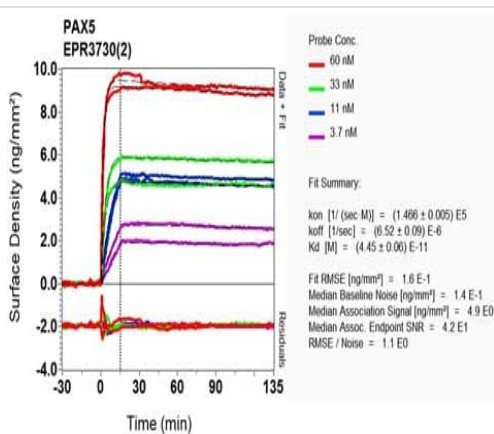
ab109443 showing negative staining in Normal human kidney tissue.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Flow Cytometry (Intracellular) - Anti-PAX5 antibody [EPR3730(2)] (ab109443)

Overlay histogram showing Ramos cells stained with unpurified ab109443 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (unpurified ab109443, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-rabbit IgG (H+L) (ab96899) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1µg/1x10⁶ cells) used under the same conditions. Acquisition of >5,000 events was performed.







OI-RD Scanning - Anti-PAX5 antibody [EPR3730(2)] (ab109443)

Equilibrium dissociation constant (K_D)

Learn more about K_D

[Click here to learn more about \$K_D\$](#)

Why choose a recombinant antibody?

 <p>Research with confidence Consistent and reproducible results</p>	 <p>Long-term and scalable supply Recombinant technology</p>
 <p>Success from the first experiment Confirmed specificity</p>	 <p>Ethical standards compliant Animal-free production</p>

Anti-PAX5 antibody [EPR3730(2)] (ab109443)

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

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