

PAX5 Monoclonal Antibody (SP34)

Catalog NumberMA5-14585

Product data sheet

| Details | | Species Reactivity | |
|--------------------|--|--|-----------------------------|
| Size | 1 mL | Species reactivity | Human, Rat |
| Host/Isotope | Rabbit / IgG | Published species | Human, Not Applicable |
| Class | Monoclonal | Tested Applications | |
| Type | Antibody | ChIP assay (ChIP) | Dilution *2.5 µg/10^6 cells |
| Clone | SP34 | Flow Cytometry (Flow) | 1:20 |
| Immunogen | Synthetic peptide derived from the C-terminus of human Pax-5 protein | Immunohistochemistry (Paraffin) (IHC (P)) | 1:10-1:500 |
| Conjugate | Unconjugated | Western Blot (WB) | 1:500 |
| Form | Liquid | Immunocytochemistry (ICC/IF) | 1:250 |
| Concentration | 0.067 mg/mL | Published Applications | |
| Purification | Protein A/G | Immunohistochemistry (Paraffin) (IHC (P)) | See 1 publications below |
| Storage buffer | PBS, pH 7.6, with 1% BSA | Immunohistochemistry (IHC) | See 2 publications below |
| Contains | 0.1% sodium azide | * Suggested working dilutions are given as a guide only. It is recommended that the user titrate the product for use in their own experiment using appropriate negative and positive controls. | |
| Storage Conditions | Store at 4°C short term. For long term storage, store at -20°C, avoiding freeze/thaw cycles. | | |

Product specific information

MA5-14585 targets Pax-5 in IHC (P) applications and shows reactivity with Human and Mouse samples. The MA5-14585 immunogen is synthetic peptide derived from the C-terminus of human Pax-5 protein.

Background/Target Information

PAX5 is a member of the paired box (PAX) family of transcription factors. The PAX proteins are important regulators in early development and alterations in the expression of their genes are thought to contribute to neoplastic transformation. PAX5 is the B-cell lineage specific activator protein (BSAP) that is expressed at early, but not late stages, of B-cell differentiation. Its expression has also been detected in developing CNS and testis, therefore, PAX5 may not only play an important role in B-cell differentiation, but also in neural development and spermatogenesis. Mutations in the gene can result in leukemia, acute lymphoblastic 3.

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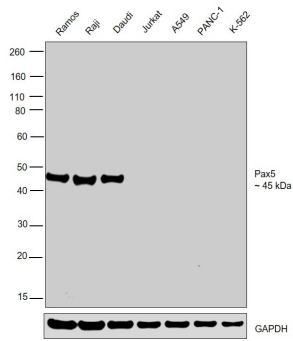
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Product Images For PAX5 Monoclonal Antibody (SP34)

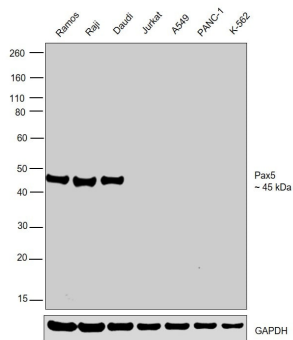
PAX5 Antibody (MA5-14585)

Antibody specificity was demonstrated by detection of differential basal expression of the target across cell lines owing to their inherent genetic constitution. The expression was observed in Ramos, Raji and Daudi and not in Jurkat, A549, PANC-1 and K-562 using PAX5 Monoclonal Antibody (SP34) (Product # MA5-14585) in Western Blot. {RE}



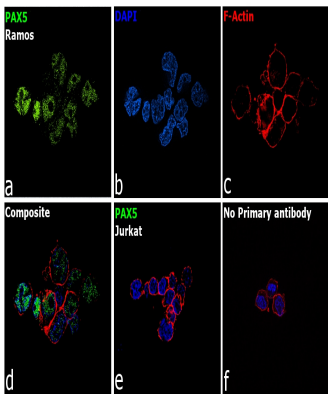
PAX5 Antibody (MA5-14585) in WB

Western blot was performed using Anti-PAX5 Monoclonal Antibody (SP34) (Product # MA5-14585) and a 45 kDa band corresponding to PAX5 was observed in Ramos, Raji and Daudi and not in Jurkat, A549, PANC-1 and K-562. Modified whole cell extracts (1% SDS) (30 µg lysate) of Ramos (Lane 1), Raji (Lane 2), Daudi (Lane 3), Jurkat (Lane 4), A549 (Lane 5), PANC-1 (Lane 6) and K-562 (Lane 7) were electrophoresed using Novex® NuPAGE® 4-12% Bis-Tris Protein Gel (Product # NP0322BOX). Resolved proteins were then transferred onto a nitrocellulose membrane (Product # IB23001) by iBlot® 2 Dry Blotting System (Product # IB21001). The blot was probed with the primary antibody (1:500 dilution) and detected by chemiluminescence with Goat anti-Rabbit IgG (Heavy Chain), Superclonal™ Recombinant Secondary Antibody, HRP (Product # A27036), using the iBright FL 1000 (Product # A32752). Chemiluminescent detection was performed using Novex® ECL Chemiluminescent Substrate Reagent Kit (Product # WP20005).



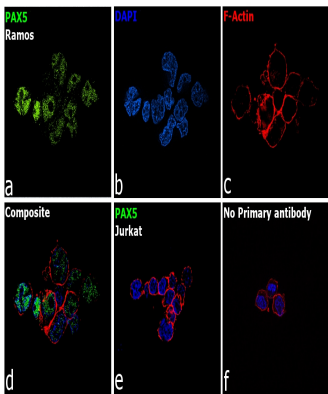
PAX5 Antibody (MA5-14585) in ICC/IF

Immunofluorescence analysis of PAX5 was performed using 70% confluent log phase Ramos cells. The cells were fixed with 4% Paraformaldehyde for 10 minutes, permeabilized with 0.1% Triton™ X-100 for 10 minutes, and blocked with 2% BSA for 10 minutes at room temperature. The cells were labeled with PAX5 Monoclonal Antibody (SP34) (Product # MA5-14585) at 1:250 dilution in 0.1% BSA, incubated at 4 degree Celsius overnight and then labeled with Goat anti-Rabbit IgG (Heavy Chain), Superclonal™ Recombinant Secondary Antibody, Alexa Fluor 488 (Product # A27034, 1:2000 dilution) for 45 minutes at room temperature (Panel a: Green). Nuclei (Panel b: Blue) were stained with SlowFade® Gold Antifade Mountant with DAPI (Product # S36938). F-actin (Panel c: Red) was stained with Rhodamine Phalloidin (Product # R415, 1:300). Panel d represents the merged image showing nuclear localization. Panel e represents Jurkat cells having no expression of PAX5. Panel f represents control cells with no primary antibody to assess background. The images were captured at 60X magnification.



PAX5 Antibody (MA5-14585)

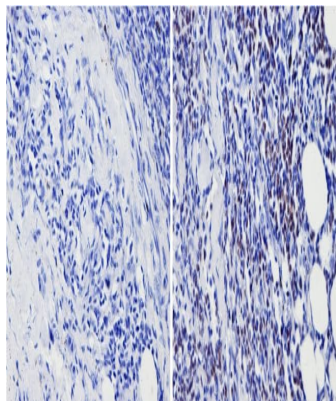
Antibody specificity was demonstrated by detection of differential basal expression of the target across cell models owing to their inherent genetic constitution. Immunofluorescence analysis using Anti-PAX5 Monoclonal Antibody (SP34) (Product # MA5-14585) shows increased expression of PAX5 in Ramos cell line when compared to Jurkat. {RE}



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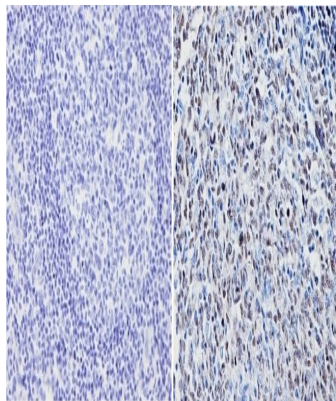
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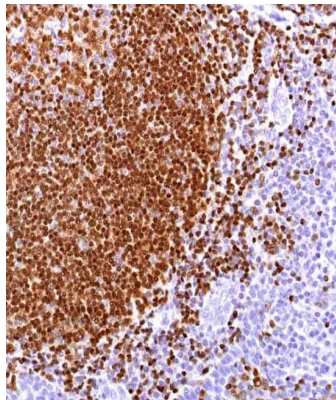
PAX5 Antibody (MA5-14585) in IHC (P)

Immunohistochemistry analysis of Pax-5 showing positive staining in the nucleus of paraffin-treated Human B lymphoma (right) compared with a negative control in the absence of primary antibody (left). To expose target proteins, antigen retrieval method was performed using 10mM sodium citrate (pH 6.0) microwaved for 8-15 min. Following antigen retrieval, tissues were blocked in 3% H₂O₂-methanol for 15 min at room temperature, washed with ddH₂O and PBS, and then probed with a Pax-5 monoclonal antibody (Product # MA5-14585) diluted by 3% BSA-PBS at a dilution of 1:20 overnight at 4°C in a humidified chamber. Tissues were washed extensively PBST and detection was performed using an HRP-conjugated secondary antibody followed by colorimetric detection using a DAB kit. Tissues were counterstained with hematoxylin and dehydrated with ethanol and xylene to prep for mounting.



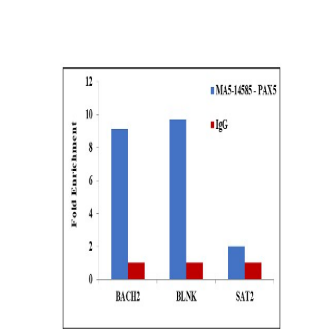
PAX5 Antibody (MA5-14585) in IHC (P)

Immunohistochemistry analysis of Pax-5 showing positive staining in the nucleus of paraffin-treated Human tonsil tissue (right) compared with a negative control in the absence of primary antibody (left). To expose target proteins, antigen retrieval method was performed using 10mM sodium citrate (pH 6.0) microwaved for 8-15 min. Following antigen retrieval, tissues were blocked in 3% H₂O₂-methanol for 15 min at room temperature, washed with ddH₂O and PBS, and then probed with a Pax-5 monoclonal antibody (Product # MA5-14585) diluted by 3% BSA-PBS at a dilution of 1:20 overnight at 4°C in a humidified chamber. Tissues were washed extensively PBST and detection was performed using an HRP-conjugated secondary antibody followed by colorimetric detection using a DAB kit. Tissues were counterstained with hematoxylin and dehydrated with ethanol and xylene to prep for mounting.



PAX5 Antibody (MA5-14585) in IHC (P)

Formalin-fixed, paraffin-embedded human Tonsil stained with PAX-5 using peroxidase-conjugate and DAB chromogen. Note nuclear staining.



PAX5 Antibody (MA5-14585) in ChIP

Chromatin Immunoprecipitation (ChIP) assay of endogenous PAX5 protein using Anti-PAX5 Antibody: ChIP was performed using Anti-PAX5 Monoclonal Antibody (Product # MA5-14585, 5 µg) on sheared chromatin from Raji cells using the MAGnify ChIP System kit (Product # 49-2024). Normal Rabbit IgG was used as a negative IP control. The purified DNA was analyzed by qPCR using primers binding to BACH2 and BLNK promoter and SAT2 satellite repeats. Data is presented as fold enrichment of the antibody signal versus the negative control IgG using the comparative CT method.

PubMed References For PAX5 Monoclonal Antibody (SP34)

1 Immunohistochemistry (Paraffin) References

| Species / Dilution | Summary |
|--------------------|--|
| Human / 1:50 | MA514585 was used in immunohistochemistry - paraffin section to develop an "antigen relaxing" method to improve routine detection of scarce antigens in formalin-fixed, paraffin-embedded material |
| | Applied immunohistochemistry & molecular morphology : AIMM (2016; 24: 436) "A 2-Step Laemmli and Antigen Retrieval Method Improves Immunodetection." Author(s):Scalia CR,Gendusa R,Cattoretti G PubMed Article URL: http://dx.doi.org/10.1097/PAI.0000000000000203 |

2 Immunohistochemistry References

| Species / Dilution | Summary |
|----------------------------|--|
| Not Applicable / Not Cited | MA5-14585 was used in immunohistochemistry to study a northern New England hospital and increased utilization, verification, and clinical implications of immunocytochemistry |
| | Diagnostic cytopathology (2015; 43: 688) "Increased utilization, verification, and clinical implications of immunocytochemistry: Experience in a northern New England hospital." Author(s):Sauter JL,Ambaye AB,Mount SL PubMed Article URL: http://dx.doi.org/10.1002/dc.23279 |
| Human / 1:100 | MA5-14585 was used in immunohistochemistry to present a case report of an unusual composite lymphoma in the mediastinum |
| | Diagnostic pathology (2011; 6:) "Composite lymphoma in the anterior mediastinum: a case report and review of the literature." Author(s):Yu G,Kong L,Qu G,Zhang Q,Wang W,Jiang L PubMed Article URL: http://dx.doi.org/10.1186/1746-1596-6-60 |

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