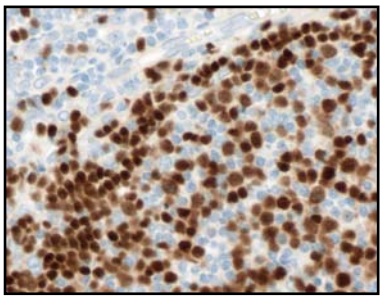
CONFIRM anti-PAX5 (SP34) Rabbit Monoclonal Primary Antibody

Catalog Number 790-4420



INTENDED USE

This antibody is intended for *in vitro* diagnostic (IVD) use. Ventana Medical Systems' (Ventana) CONFIRM anti-PAX5 (SP34) Rabbit Monoclonal Primary Antibody is directed against a nuclear epitope present in human B lymphocytes. This antibody may be used to aid in the identification of normal and neoplastic cells of B-lymphocytic lineage. The antibody is intended for qualitative staining in sections of formalin fixed, paraffin embedded tissue. The clinical interpretation of any staining, or the absence of staining, shall be complemented by morphological studies and evaluation of proper controls. Evaluation shall be made by a qualified pathologist within the context of the patient's clinical history and other diagnostic tests.

SUMMARY AND EXPLANATION

This rabbit monoclonal antibody is directed against a synthetic peptide from the C-terminus of human PAX-5 protein. PAX-5 is a B-cell-specific activator protein (BSAP).[1] In the early stages of B cell development, PAX-5 influences the expression of several B-cell-specific genes, which code for CD19 and CD20.[2] PAX-5 is expressed primarily in pro-, pre-, and mature B cells, but not in plasma cells.[2, 3] Interestingly, PAX-5 mRNA is transiently detected in the mesencephalon and spinal cord during embryogenesis. Expression then shifts to the fetal liver and correlates with the onset of B-cell lymphopoiesis.[1] This indicates that PAX-5 is important in B cell development, but may also have a role in proper neuronal cell development. PAX-5 is expressed in the majority of B-cell lymphomas without plasma cell differentiation.[4] Plasma cell neoplasms, multiple myeloma, and plasmablastic lymphomas are typically negative when stained with PAX-5.[5,6] T-cell lymphomas to date are consistently negative.[4]

REAGENT PROVIDED

CONFIRM anti-PAX5 (SP34) contains sufficient reagent for staining 50 slides.

One 5 mL dispenser of CONFIRM anti-PAX5 (SP34) contains approximately 5 µg of a recombinant rabbit monoclonal antibody.

The antibody is diluted in 0.05 M Tris-HCl with 1% carrier protein, and 0.10% ProClin300, a preservative.

Total protein concentration of the reagent is approximately 10 mg/mL. Specific antibody concentration is approximately 1.0 µg/mL. There is no known irrelevant antibody reactivity observed in this product.

Refer to the appropriate Ventana detection kit package insert for detailed descriptions of: (1) Principles of the Procedure, (2) Materials and Reagents Needed but Not Provided, (3) Specimen Preparation, (4) Quality Control, (5) Troubleshooting, (6) Interpretation of Staining, and (7) General Limitations.

MATERIALS REQUIRED BUT NOT PROVIDED

Staining reagents such as Ventana detection kits (for example, *ultra*View Universal DAB detection kit) and ancillary components, including negative and positive tissue control slides, are not provided.

STORAGE

Store at 2 – 8ºC. Do not freeze.

To ensure proper reagent delivery and stability of the antibody, after every use the cap must be replaced and the dispenser must be immediately placed in the refrigerator in an upright position.

Every antibody dispenser is expiration dated. When properly stored, the reagent is stable to the date indicated on the label. Do not use reagent beyond the expiration date.

SPECIMEN PREPARATION

Routinely processed, formalin fixed, paraffin embedded tissues are suitable for use with this primary antibody when used with Ventana detection kits and a Ventana automated slide stainer. The recommended tissue fixative is 10% neutral buffered formalin.[7] Slides should be stained immediately, as antigenicity of cut tissue sections may diminish over time.

It is recommended that positive and negative controls be run simultaneously with unknown specimens.

WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic use.
2. This product contains 1% or less bovine serum which is used in the manufacture of the antibody.
3. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
4. Avoid microbial contamination of reagents.
5. Consult local or state authorities with regard to recommended method of disposal.

STAINING PROCEDURE

Ventana primary antibodies have been developed for use on a Ventana automated slide stainer in combination with Ventana detection kits and accessories. A recommended staining protocol for BenchMark series automated slide stainers with *ultra*View Universal DAB detection kit is listed below in Table 1. The parameters for the automated procedures can be displayed, printed, and edited according to the procedure in the instrument operator manual. Refer to the appropriate Ventana detection package insert for more details regarding immunohistochemistry staining procedures.

Table 1. Recommended Staining Protocol for CONFIRM anti-PAX5 (SP34) with *ultra*View Universal DAB detection kit on BenchMark series automated slide stainers

Procedure Type	Method
Deparaffinization	Selected
Cell Conditioning (Antigen Unmasking)	Standard Cell Conditioning 1
Enzyme (Protease)	None required
Antibody (Primary) Incubation	BenchMark XT instrument Approximately 16 Minutes, 37°C BenchMark ULTRA instrument Approximately 32 Minutes, 36°C
Counterstain	Hematoxylin II, 2 to 4 Minutes
Post Counterstain	Bluing Reagent, 2 to 4 Minutes

Due to variation in tissue fixation and processing, as well as general lab instrument and environment conditions, it may be necessary to increase or decrease the primary antibody incubation and cell conditioning based on individual specimens, detection used, and reader preferences. For further information on fixation variables, refer to "Immunohistochemistry Principles and Advances" .[8]

POSITIVE TISSUE CONTROL

Examples of positive control tissues for CONFIRM anti-PAX5 (SP34) are normal tonsil (as depicted in the above image) or spleen.

STAINING INTERPRETATION

The cellular staining pattern for CONFIRM anti-PAX5 (SP34) is nuclear.

SPECIFIC LIMITATIONS

This antibody has been optimized for a 16 minute incubation time on BenchMark XT instruments, or a 32 minute incubation time on BenchMark ULTRA instruments in combination with *ultra*View Universal DAB detection kit, however the user must validate results obtained with this reagent. Bone Marrow specimens tested successfully with this antibody were all prepared in B5 fixative.

PERFORMANCE CHARACTERISTICS

1. Specificity of CONFIRM anti-PAX5 (SP34) was determined by testing formalin fixed, paraffin embedded normal and neoplastic tissues. For normal tissues, results are as follows: Adrenal gland (0/3), bone marrow in B5 fixative (5/5), brain cerebrum (0/3),

brain cerebellum (0/3), breast (0/3), cervix (0/3), colon (0/3), esophagus (0/3), heart (0/3), hypophysis (0/3), intestine (1/3), kidney (0/3), liver (0/3), lung (0/3), reactive hyperplastic lymph node (8/10), mesothelium (0/3), nerve (0/3), ovary (0/3), pancreas (0/3), parathyroid (0/3), prostate (0/3), salivary gland (0/3), skin (0/3), spleen (3/3), stomach (0/3), striated muscle (0/3), testis (0/3), thymus (1/3), thyroid (0/3), tonsil (2/3), and uterus (0/3). For neoplastic tissues, results are as follows: Atypical meningioma (0/1), glioblastoma (0/1), ependymoma (0/1), oligodendroglioma (0/1), ovarian serous papillary adenocarcinoma (0/1), ovarian mucous papillary adenocarcinoma (0/1), islet cell carcinoma (0/1), pancreatic adenocarcinoma (0/1), testicular seminoma and embryonal carcinoma (0/2), medullary thyroid carcinoma (0/1), papillary thyroid carcinoma (0/1), breast carcinoma (0/3), diffuse large B-cell lymphoma (56/60), follicular lymphoma (7/7), mantle cell lymphoma (4/4), extranodal marginal zone B-cell lymphoma (2/2), musoca-associated lymphoma (11/14), lymphoblastic lymphoma (2/3), precursor lymphoblastic lymphoma (0/1), precursor B-cell lymphoblastic lymphoma (1/1), granulocytic sarcoma (0/1), anaplastic large cell lymphoma (2/2), LCL plasmablastic (0/1), small cell lymphoma (4/7), T-cell lymphoma (0/15), lung carcinoma (0/3), esophageal squamous cell and adenocarcinoma (0/2), adenocarcinoma in stomach (0/1), intestinal adenocarcinoma (0/2), colorectal adenocarcinoma (0/4), hepatocellular carcinoma (0/1), hepatoblastoma (0/1), clear cell carcinoma (0/1), adenocarcinoma in prostate (0/1), transitional cell carcinoma in prostate (0/1), uterine leiomyoma (0/1), endometrial carcinoma (0/1), uterine clear cell and squamous carcinomas (0/3), embryonal rhabdomyosarcoma (0/1), rectal melanoma (0/1), basal cell carcinoma in skin (0/1), squamous cell carcinoma in skin (0/1), neurofibroma and neuroblastoma (0/2), malignant mesothelioma (0/1), Hodgkin's lymphoma (2/3), bladder transitional cell carcinoma (0/1) leiomyosarcoma (0/2), osteosarcoma (0/1), and spindle cell rhabdomyosarcoma (0/1).

2. Lot to lot reproducibility was determined by testing 3 lots across 1 multi-tissue block (3 tissues per block) on a BenchMark XT instrument. 3 out of 3 samples across all 3 lots scored equivalently.
3. Inter-run repeatability was determined by staining 2 multi-tissue blocks (3 tissues per block for a total of 6 tissues) across 5 slides on a BenchMark XT instrument over a five day non-consecutive period. 150 out of 150 samples tested scored equivalently.
4. Intra-run repeatability was determined by staining 2 multi tissue blocks (3 tissues per block for a total of 6 tissues) across 14 slides on a BenchMark XT instrument. 84 out of 84 samples tested scored equivalently.
5. Intra-platform repeatability was determined by staining 2 multi-tissue blocks (3 tissues per block) across 5 slides on 3 BenchMark XT instruments. 89 out of 90 samples tested scored equivalently.
6. Intra-platform repeatability was determined by staining 1 multi-tissue block (3 tissues) across 5 slides on 3 BenchMark ULTRA instruments. 45 out of 45 samples tested scored equivalently.
7. Inter-platform repeatability was determined by staining 1 multi-tissue block (3 tissues per block) across 5 slides on 3 BenchMark XT instruments and 3 BenchMark ULTRA instruments. 90 out of 90 samples tested scored equivalently.
8. Compatible with iVIEW DAB and *ultra*View Universal DAB detection kits.

REFERENCES

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8. Roche PC, and E.D. Hsi. Immunohistochemistry-Principles and Advances. Manual of Clinical Laboratory Immunology, 6th edition. (NR Rose Ed.) ASM Press, 2002.

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