NAME: Monoclonal Mouse Anti-Human T-cell, CD45RO, Clone UCHL1

IMMUNOGEN: IL-2 dependent T-cell line CA1

CLONE/REF: UCHL1

CLASS/SUBCLASS: IgG2a, Kappa

CODE NO.: N1520

Ready-to-Use DAKO® N-SERIES Primary Antibody and Negative Control Reagent

For use with DAKO EnVision™, DAKO EnVision™ Doublestain and DAKO LSAB®2 Systems

INTENDED USE:

For In Vitro Diagnostic Use.

This antibody is intended for laboratory use to identify qualitatively by light microscopy T-cells in normal and neoplastic tissues using immunohistochemical (IHC) test methods. Positive results aid in the classification of lymphomas as T-cell in origin. Differential identification is aided by the results from a panel of antibodies. Interpretation must be made within the context of the patient’s clinical history and other diagnostic tests by a qualified pathologist.

Refer to the “General Instructions for Immunohistochemical Staining” or the Detection System “Instructions” of IHC procedures for:


SUMMARY AND EXPLANATION:
The Monoclonal Mouse Anti-Human T-cell, CD45RO, Clone UCHL1 (Anti-CD45RO, UCHL1) antibody recognizes a 180 kDa isoform of the leucocyte common antigen (CD45) family.1-3 The antibody was designated as belonging to the CD45RO family at the Fourth International Workshop on Human Leucocyte Differentiation Antigens (Vienna, 1989).2 The CD45RO antigen occurs on most thymocytes and activated T-cells, but only on a proportion of resting T-cells. It is widely regarded as a marker for a distinct population of T-cells that are stimulated by soluble antigens to proliferate at a high rate and includes memory T-cells.1,4,5

REAGENTS PROVIDED:
The primary antibody is available in 7 mL and 22 mL sizes as tissue culture supernatant in 0.05 mol/L Tris-HCl, pH 7.6, containing stabilizing protein and 0.015 mol/L sodium azide.

The negative control reagent is available in 5 mL and 11 mL sizes as fetal calf serum in 0.05 mol/L Tris-HCl, pH 7.6, containing stabilizing protein and 0.015 mol/L sodium azide.

This product has been optimized for use in DAKO EnVision™, DAKO EnVision™ Doublestain and DAKO LSAB®2 detection systems. This product has not been tested with detection systems other than those listed. The primary antibody and negative control reagents should be applied as directed in the Staining Procedure section of the Instructions included with each detection system. The recommended incubation time for this primary antibody is 10 minutes at room temperature.

MATERIALS REQUIRED, NOT SUPPLIED:

Refer to the “General Instructions for Immunohistochemical Staining” and/or the Detection System “Instructions”.

PRECAUTIONS:

1. For In Vitro Diagnostic Use.
2. This product contains sodium azide (NaN₃), a chemical highly toxic in pure form. At product concentrations, though not classified as hazardous, build-ups of NaN₃ may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with large volumes of water to prevent azide build-up in plumbing.6
3. Minimize microbial contamination of reagents or increase in nonspecific staining may occur.

STORAGE:

Store at 2-8°C.

Do not use after expiration date stamped on vial. If reagents are stored under any conditions other than those specified, the conditions must be verified by the user.7

There are no obvious signs to indicate instability of this product. Therefore, positive and negative controls should be run simultaneously with patient specimens. If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem with the antibody is suspected, contact DAKO Technical Services.

SPECIMEN PREPARATION:

Paraffin Sections: Anti-CD45RO, UCHL1 can be used on formalin-fixed, paraffin-embedded tissue sections. Other preservatives that are compatible with anti-CD45RO, UCHL1 include neutral-buffered or unbuffered formalin, zinc-formalin or B5 fixative. Pretreatment of tissue with proteolytic enzymes is not recommended.

Cryostat Sections And Cell Smears: Anti-CD45RO, UCHL1 can be used for labelling acetone-fixed cryostat sections or fixed cell smears.

STAINING PROCEDURE:

Follow the procedure for the detection system selected. Use the recommended incubation time presented in the “Reagents Provided” section above.
Abnormal tissues: Anti-CD45RO, UCHL1 cross-reactivity with myeloid lineage cells has also been noted with mature myeloid cells in granulocytic sarcoma; and macrophages in histiocytic lymphomas, malignant histiocytosis of the intestine, and Langerhans cell histiocytosis. Morphological studies and a panel of antibodies which includes anti-CD45RO, UCHL1 are recommended for use in the differential diagnosis of granulocytic sarcoma, plasmacytoma, and histiocytic neoplasia from T-cell lymphomas.

PRODUCT SPECIFIC LIMITATIONS:
1. It is important to differentiate between the ring-like membrane staining pattern associated with a T-cell lineage and the nonspecific cytoplasmic and nuclear staining. Nuclear staining may represent cross-reactivity with an unknown nuclear antigen. No other anti-CD45RO, UCHL1 reactivity has been noted with normal or malignant non-lymphoid cells.
2. The influence of the type of fixative is controversial. Linder, et al. reported 97% immunoreactivity with T-cell lymphomas fixed in B5 whereas 50% immunoreactivity was observed in the same T-cell lymphomas fixed in 10% neutral-buffered formalin. Others report that mercurial fixatives are unsuitable at preserving the membrane antigen.
3. Staining has been detected in select groups of B-cell pathological tissues. A few cases of diffuse large cell non-Hodgkin’s B-cell lymphomas (8/50, 18%) coexpress the CD45RO antigen.
4. One group has reported weak membrane staining in scattered Reed-Sternberg cells in some cases of Hodgkin’s Disease (8/32, 25%), while other groups reported no staining of Reed-Sternberg cells.
5. One group has reported weak membrane staining in scattered Reed-Sternberg cells in some cases of Hodgkin’s Disease (8/32, 25%), while other groups reported no staining of Reed-Sternberg cells.
6. Cells of the myelomonocytic series are also labelled by urinary bladder and uterus. Nonspecific cytoplasmic staining has been reported in smooth muscle, hepatocytes, squamous and transitional epithelium, gall bladder and breast tissue.
7. The cellular staining pattern for anti-CD45RO, UCHL1 is membranous. Positive anti-CD45RO, UCHL1 staining does not exclude T-cell pathogenesis. This antibody is most effective when used in a panel of antibodies.

REFERENCES:
15. Herman GE, Eiffet E, Aberant CG45 (leukocyte common antigen) staining of non-malignant breast lesions in zinc formalin fixed tissue. J Histotechnol 1993; 16(2):151