



## CD45RO

### Monoclonal Mouse Anti-CD45RO

#### Clone UCHL-1

**REF** 014-1012

**Ready-To-Use** ■ 100 Tests/50 Tests

**Concentrate** ■ 1mL

#### INTENDED USE

**IVD** For in vitro diagnostic use.

Celerus monoclonal mouse anti-CD45RO, clone UCHL-1, is intended for laboratory use in identifying T cells in normal or pathological tissue using light microscopy. It may be used with frozen tissue or with formalin-fixed paraffin-embedded tissue.

Positive results aid in the classification of normal and abnormal cells/tissues and serve as an adjunct to conventional histopathology. The clinical interpretation of any positive staining or its absence should be complemented by morphological and histological studies with proper controls. Evaluations should be made by a qualified individual in conjunction with the patient's clinical history and other diagnostic test results.

Refer to the Wave Instrument Operator's Manual for additional information concerning Materials Required but Not Provided; Storage; Staining Procedure; Troubleshooting; Interpretation of Staining; and General Limitations.

#### SUMMARY AND EXPLANATION

The CD45RO molecule, a 180kD isoform of CD45, is expressed on 48 percent of peripheral blood T lymphocytes, 37 per cent of CD4 positive lymphocytes, and 80 percent of thymocytes. The antigen is present on the surface of monocytes and granulocytes but in the cytoplasm of macrophages.

This antibody reacts with most normal thymocytes, granulocytes, and macrophages, and with most T cell malignancies.

#### PRINCIPLE OF PROCEDURE

Immunohistochemistry is a multi-step process to identify specific cell markers within tissue biopsies or tumor specimens. The sequential steps include antigen retrieval (optional), antibody application, and antibody visualization followed by optional counterstaining. Specimens are then coverslipped and observed under light microscopy by trained personnel. Normally, multiple antibodies are tested to determine lineage and cell cycle markers. The Celerus Wave is an automated instrument that performs immunohistochemistry stains. For further information on the staining procedure, refer to the Celerus Wave Operator's Manual.

#### MATERIALS AND METHODS

##### Reagent Provided

Clone  
UCHL-1

##### Ig Class

IgG2<sub>a</sub>

##### Immunogen

IL-2 dependent T-cell line (CA1).

##### Ready-To-Use in Primary Antibody Cartridge

Celerus anti-CD45RO is provided with ProClin 300 as a preservative, in a Primary Antibody Cartridge (PAC), a self-contained dispenser of reagents. Each PAC contains sufficient reagent to complete 50 or 100 stained slides. PACs must remain upright to avoid spilling. PAC must be primed before first use. See Celerus Wave Operator's Manual for details.

#### Concentrated Antibody

##### Liquid

Liquid concentrated antibody is provided containing 15 mM sodium azide as a preservative and 1% bovine serum albumin as a carrier protein.

##### Lyophilized

Lyophilized antibody is provided containing 15mM sodium azide as a preservative. Reconstitute vial with 1.0 ml distilled water.

##### Dilution

The suggested dilution is 1:50-1:100. This is a guide only and users should determine their own optimal working dilutions.

#### SPECIFICITY

Human CD45RO antigen.

#### MATERIALS REQUIRED BUT NOT PROVIDED

- Wave instrument
- Wave slide rack
- Positively-charged microscope slides, appropriately labeled
- Timer
- Celerus Riptide for antigen retrieval (or equivalent)
- Celerus Target Retrieval Solution (or equivalent)
- Slide drying chamber
- Xylene or xylene substitute
- Reagent alcohol or ethyl alcohol
- Distilled or deionized water
- TBS wash buffer, pH 7.6
- Positive and negative tissue controls
- Celerus Negative Control Reagent (or equivalent)
- Mounting Medium
- Cover slips

#### STORAGE AND HANDLING

##### Ready-to-Use PAC, Liquid Concentrated and Lyophilized Antibody

Store reagent at 2-8 °C. Do not freeze. The reagent is stable until the expiration date on the container. Do not use reagent after the expiration date, as the activity cannot be ensured.

##### Reconstituted Antibody

For reconstituted antibody, the reagent is stable for at least two months when stored at 4 °C. For long-term storage it is recommended that aliquots of the antibody be stored at -20 °C. Repeated freezing and thawing of the antibody should be avoided.

There are no signs to indicate instability of this reagent. To ensure a valid staining assay, the use of positive and negative tissue controls is recommended. Contact your Celerus representative if there are stability concerns prior to the expiration date.

#### PRECAUTIONS

- For professional users.
- Minimize microbial contamination of reagents or an increase in nonspecific staining may occur.
- As with any product derived from biological sources, proper handling procedures should be used.
- A Material Safety Data Sheet is available for professional users on request.

- ProClin 300 is classified per applicable European Community (EC) Directives as: Irritant (Xi). The following are the appropriate Risk (R) and Safety (S) phrases.



- R36** Irritating to eyes  
**R43** May cause sensitization by skin contact  
**S24** Avoid contact with skin  
**S26** In case of contact with eyes, rinse immediately with plenty of water and seek medical advice  
**S35** This material and its container must be disposed of in a safe way  
**S37/39** Wear suitable gloves and eye/face protection  
**S46** If swallowed, seek medical advice immediately and show this container or label.

#### WASTE DISPOSAL

Adhere to all local laws when disposing of the PAC.

#### PACKAGING DAMAGE

DO NOT USE a PAC if it is leaking, has leaked, has spilled, cannot be primed, or has visually apparent physical damage.

#### SPECIMEN COLLECTION AND HANDLING

Formalin-fixed paraffin embedded (FFPE) tissues, frozen tissues, or smears are suitable for use. Wave detection kits have been optimized for tissues fixed with 10% formalin. Ideally, each 4-6µ tissue section should be placed on charged slides on the lower 2/3 of the slide. Very large sections should be placed 1/4 inch below the lower end of the slide label.

Slides should be baked overnight at 37 °C, or at 60 °C for one hour.

Use standard histochemical techniques to deparaffinize processed slides. For uniformity of staining results, it is recommended that target retrieval be performed using the Celerus Riptide and Celerus Target Retrieval Solution (or equivalent) at 112 °C for 5 minutes. Insert slides into the Wave Instrument according to the staining grid provided by the instrument software. Avoid drying of the tissue specimen during this process. After all slides to be stained have been inserted and reagents mounted on the instrument, start the staining run.

When the slides have completed the staining run, remove them from the instrument, coverslip, and view under light microscopy.

#### PRODUCT-SPECIFIC LIMITATIONS

CD45RO antibody, when used on the Wave instrument, detects antigens that survive routine formalin fixation, tissue

processing, and sectioning. Users who deviate from recommended test procedures are responsible for interpretation and validation of patient results.

#### RESULTS EXPECTED/ PERFORMANCE CHARACTERISTICS

##### Normal Tissues

The CD45RO antibody reacts with most T lymphocytes, macrophages, and Langerhan's cells of normal tissue.

##### Abnormal Tissues

Positive staining occurred in 72-84% of paraffin-embedded T-cell lymphomas. Negative staining was more frequently seen on T-cell derived lymphoblastic lymphomas. During T cell activation, expression of the CD45RO antigen increases, which may explain the difference in the immunoreactivity of some T-cell lymphomas.

A panel of antibodies should be used for the differential diagnosis of granulocytic sarcoma, plasmacytoma, and histiocytic neoplasia from T-cell lymphomas.

#### REFERENCES

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