P.O. Box 3286 - Logan, Utah 84323, U.S.A. - Tel. (800) 729-8350 – Tel. (435) 755-9848 - Fax (435) 755-0015 - www.ScyTek.com

CD45RA (LCA); Clone 158-4D3

Catalog Number	Format	Volume
A00123-0002	(Ready-To-Use)	2 ml
A00123-0007	(Ready-To-Use)	7 ml
A00123-0025	(Ready-To-Use)	25 ml
A00123-C	(Concentrate)	1 ml

Intended Use

For In-Vitro Diagnostic Use. This antibody is intended for the qualitative visualization of the anatomical elements listed in the Specificity section. It is intended to be used within an Immunohistochemistry (IHC) procedure on formalin-fixed paraffin-embedded (FFPE) human tissue followed by visualization by light microscopy.

Description

Titer/Working Dilution: Ready-to-Use: No further dilution required.
Concentrate: Suggested dilution is 1:125-250

Species: Mouse

Immunogen: Stimulated human leukocytes were used as immunogen to generate the CD45RA antibody.

Clone: 158-4D3

Isotype: Mouse IgG2a, Kappa

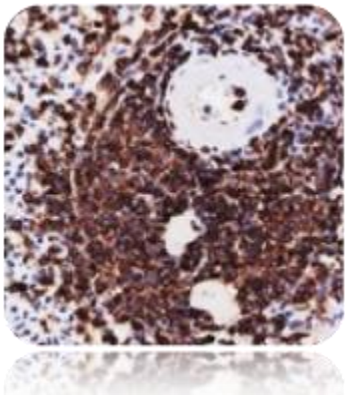
Mol. Wt. of Antigen: 205-220kDa

Format: Ready-To-Use antibody has been pre-titered and quality controlled to work on formalin-fixed paraffin-embedded as well as acetone fixed cryostat tissue sections. No further titration is required.
Concentrate antibody is provided in a phosphate buffered saline containing 1% BSA.

Specificity: The CD45RA 158-4D3 antibody clone reacts with the ABC and BC isoforms (Schlossman et al, 1995). CD45RA has a molecular weight of 205-220 kDa.

Background: CD45 (leukocyte common antigen), the most common hematopoietic lineage marker, is a transmembrane glycoprotein tyrosine phosphatase with important roles in immune system signal transduction pathways. Antibody panels have been instrumental in identifying multiple isoforms, including CD45RA. Isoforms differ in their extracellular domain and are generated by alternative splicing of exons 4, 5 and 6 encoding peptide segments called A, B, and C, respectively. At least five different isoforms have been identified in humans: containing all three (ABC), two (AB and BC), one (B) or no (O) exons. A given antibody recognizes either every isoform (CD45 antibody) or only a subset ("restricted" CD45R antibody). The CD45RA 158-4D3 antibody clone reacts with the ABC and BC isoforms (Schlossman et al, 1995). CD45RA has a molecular weight of 205-220 kDa.

Isoform expression is regulated in lymphocyte type and activation-state dependent manners. CD45RA is expressed on subsets of T and B cells rendering the CD45RA antibody particularly useful for studying subpopulations. For example, naïve T lymphocytes express CD45RA containing the A exon which is lost after activation and replaced by CD45R0. In this regard, the CD45RA antibody is a useful naïve T cells marker since they express CD45RA, and activated or memory T cells are CD45RA negative and CD45RO positive (Schlossman et al, 1995). CD45RA is differentially expressed on lymphomas and the CD45RA antibody may also be used to help identify or classify lymphomas such as the CD45RA positive B from the CD45R0 positive T cell lymphomas. Researchers are encouraged to consult the scientific literature for additional information about CD45RA expression, including the use of antibody panels that include a CD45RA antibody for characterizing a given malignancy.



Species Reactivity: Human. Others not known.
Positive Control: Tonsil, Spleen.
Microbiological State: Nonsterile

Materials and Reagents Required but not Provided

1. Control tissue and reagents
2. Xylene, graded alcohols, and deionized/distilled water
3. Antibody Diluent.
4. IHC detection system. Suggested: ScyTek Cat# ABZ125 "CRF Anti-Polyvalent HRP Polymer" and ScyTek Cat# ACV500 "DAB Chromogen/Substrate Kit (High Contrast)".
5. Wash buffer for rinses (ScyTek Cat# TBT500)
6. HIER Retrieval Solution
7. Hematoxylin counterstain and bluing reagent (ScyTek Cat# HMM500 and BRT500)
8. Mounting medium and coverslips

Note: ScyTek Laboratories has a wide range of IHC reagents and ancillaries that can be found at scytek.com.

Procedure

1. **Tissue Section Pretreatment (Highly Recommended):** Staining of formalin fixed paraffin embedded tissue sections is significantly enhanced by pretreatment with Tis-EDTA HIER Solution (10x) pH 9.0 (ScyTek catalog# TES500) or Citrate Plus (10x) HIER Solution (ScyTek catalog# CPL500)

2. **Primary Antibody Incubation Time:** We suggest an incubation period of 30 minutes at room temperature. However, depending upon the fixation conditions and the staining system employed, optimal incubation should be determined by the user.

3. **Visualization:** For maximum staining intensity we recommend the "CRF Anti-Polyvalent HRP Polymer" (ScyTek catalog# ABZ125, see IFU for instructions) combined

Storage: 2° C 8° C

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with the "DAB Chromogen/Substrate Bulk Pack (High Contrast)" (ScyTek catalog# ACV500, see IFU for instructions).

Storage and Stability

Do not Freeze. Store at 2-8°C. Return to 2-8° immediately after use. Do not use after expiration date printed on label. Verify visually that antibody has not been contaminated before use. Do not use if reagent becomes cloudy or precipitates.

Limitations

Immunohistochemistry is a complex technique involving both histological and immunological detection methods. Tissue processing and handling prior to immunostaining can cause inconsistent results. Variations in fixation and embedding or the inherent nature of the tissue specimen may cause variations in results. Endogenous peroxidase activity or pseudoperoxidase activity in erythrocytes and endogenous biotin may cause non-specific staining depending on detection system used. This data sheet's recommendations and procedures were validated using ScyTek IHC reagents and may not be suitable for other detection systems.

Precautions

1. Contains Sodium Azide as a preservative (0.09% w/v), do not ingest. Sodium Azide 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. This product contains no hazardous material at a reportable concentration according to U.S. 29 CFR 1910.1200, OSHA Hazardous Communication Standard and EC Directive 91/155/EC.
2. Do not pipette by mouth.
3. Avoid contact of reagents and specimens with skin and mucous membranes.
4. Avoid microbial contamination of reagents or increased nonspecific staining may occur.
5. The user must validate any procedures and recommendations that differ from this data sheet.
6. The SDS may be found at scytek.com

References

1. Shin H-M et al. Immune Network 11:114-122 (2011).
2. Clement LT. J Clin Immunol 12:1-10 (1992).
3. Saunders AE, P Johnson. Cellular Signaling 22:339-348 (2010)
4. Jacob MC et al. Am J Hematol 39:45-51 (1992).
5. Mahalingam M et al. Clin Immunol Immunopathol 81:210-214 (1996).
6. Schlossman S et al. Leukocyte Type V, Oxford University Press, Oxford, 511-515 (1995).
7. Yamada et al. Cell Immunol 142:210-214 (1992).

Warranty

No products or "Instructions For Use (IFU)" are to be construed as a recommendation for use in violation of any patents. We make no representations, warranties or assurances as to the accuracy or completeness of information provided on our IFU or website. Our warranty is limited to the actual price paid for the product. ScyTek Laboratories, Inc. is not liable for any property damage, personal injury, time or effort or economic loss caused by our products.

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