Novocastra™ Origin™ Mouse Monoclonal Antibody CD8 (Clone 1A5)

Product Code: ORG-8936

Intended Use

For in vitro diagnostic use.

CD8 (Clone 1A5) ORG-8936 is intended for the qualitative identification by light microscopy of human CD8 antigen in paraffin sections on the Ventana® Medical Systems Automated Immunohistochemistry Staining Systems. The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

Principle of Procedure

Immunohistochemical (IHC) staining techniques allow for the visualization of antigens via the sequential application of a specific antibody to the antigen (primary antibody), a secondary antibody to the primary antibody and an enzyme complex with a chromogenic substrate with interposed washing steps. The enzymatic activation of the chromogen results in a visible reaction product at the antigen site. The specimen may then be counterstained and coverslipped. Results are interpreted using a light microscope and aid in the differential diagnosis of pathophysiological processes, which may or may not be associated with a particular antigen.

Clone

1A5

Immunogen

Peptide corresponding to the alpha chain cytoplasmic portion of the human CD8 molecule.

Specificity

Human CD8 antigen

Reagent Composition

CD8 (Clone 1A5) ORG-8936 is a ready to use mouse monoclonal presented in Tris buffer pH 7.7 with 10 mg/ml carrier protein and 0.1% ProClin® 300.

Reconstitution, mixing, dilution or titration is not recommended. Further dilution may result in loss of antigen staining and must be validated by the user.

No of tests

50

Ig Class

IgG1

Total Protein Concentration

Approx 10 mg/ml

Antibody Concentration

Approx 21 µg/ml

Storage and Stability

Store antibody at 2–8 °C. Under these conditions, there is no significant loss in product performance up to the expiry date indicated on the vial label. Do not use after expiration date indicated on the vial label. Return to 2–8 °C immediately after use. Storage conditions other than those specified above must be verified by the user[1].

Specimen Preparation

The recommended fixative is 10% neutral-buffered formalin for paraffin-embedded tissue sections.

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Warnings and Precautions

Inspect vial on receipt – if damaged, do not use.

This reagent is a biological product; reasonable care should be taken when handling it. ProClin® 300 may cause irritation to skin, eyes, mucus membranes and upper respiratory tract. The concentration of ProClin® 300 in this product is 0.1% and so does not meet the OSHA criteria for a hazardous substance.

Consult federal, state or local regulations for disposal of any potentially toxic components.

Specimens, before and after fixation and all materials exposed to them, should be handled as if capable of transmitting infection and disposed of with proper precautions[2]. Never pipette reagents by mouth and avoid contacting the skin and mucus membranes with reagents and specimens. If reagents or specimens come in contact with sensitive areas, wash with copious amounts of water. Seek medical advice.

Minimize microbial contamination of reagents or an increase in non-specific staining may occur.

Incubation times or temperatures, other than those specified, may give erroneous results. Any such changes must be validated by the user.

Recommendations On Use

Prior to undertaking this methodology, users must be trained in Immunohistochemical techniques on Ventana® Medical Systems Automated Immunostainers.

Origin™ Antibodies were developed for use with the Ventana® Medical Systems, NexES® and BenchMark™ Immunohistochemistry Staining Systems in combination with Ventana® Detection Kits and Ventana® Prep Kit Dispensers. Novocastra™ has titered and quality controlled this antibody to ensure consistent and reliable performance. Before use Origin™ Antibody must be loaded into a Ventana® Prep Kit Dispenser – see the instructions supplied with Ventana® Prep Kit Dispenser. Apply the expiry date as indicted on the Origin™ Antibody vial label.

Table 1 summarises the requirements for Immunostaining.

Table 1

Requirements for FFPE Tissues	Specification - BenchMark™	Specification - NexES®
HIER (Heat Induced Epitope Retrieval) on NexES® or BenchMark™	CC1 solution	To be determined by user
Enzyme Digestion	n/a	n/a
Enzyme Incubation	n/a	n/a
Primary Antibody Incubation	16–32 minutes	16–32 minutes
Amplification	Optional	Optional

The combination of Origin™ Antibody incubation time and optimum conditions for epitope retrieval, together with the detection system should be validated by the user on a series of known positive and negative controls.

Materials required but not supplied:

1. Standard solvents used in Immunohistochemistry
2. PBS buffer
3. Retrieval solutions (if needed for HIER)
4. Ventana® NexES® or BenchMark™ Automated Staining Systems
   - Bar code labels
   - Ventana® Prep Kit Dispenser
   - Cell conditioning fluids
   - Detection kit
   - Wash solution
   - Negative control reagent
   - Liquid coverslip
5. Mounting solution and coverslips

Quality Control

Differences in tissue processing and technical procedures in the user's laboratory may produce significant variability in results, necessitating regular performance of in-house controls in addition to the following procedures.

Controls should be fresh autopsy/biopsy/surgical specimens, formalin-fixed, processed and paraffin wax-embedded as soon as possible in the same manner as the patient sample(s).

Positive Tissue Control

Used to indicate correctly prepared tissues and proper staining techniques.

One positive tissue control should be included for each set of test conditions in each staining run.

A tissue with weak positive staining is more suitable than a tissue with strong positive staining for optimal quality control and to detect minor levels of reagent degradation.[3]

Recommended positive control tissue is tonsil.

If the positive tissue control fails to demonstrate positive staining, results with the test specimens should be considered invalid.

Negative Tissue Control

Should be examined after the positive tissue control to verify the specificity of the labeling of the target antigen by the primary antibody.

Recommended negative control tissue is cerebellum.

Alternatively, the variety of different cell types present in most tissue sections frequently offers negative control sites, but this should be verified by the user.

Non-specific staining, if present, usually has a diffuse appearance. Sporadic staining of connective tissue may also be observed in sections from excessively formalin-fixed tissues. Use intact cells for interpretation of staining results. Necrotic or degenerated cells often stain non-specifically.[4] False-positive results may be seen due to non-immunological binding of proteins or substrate reaction products. They may also be caused by endogenous enzymes such as pseudoperoxidase (erythrocytes), endogenous peroxidase (cytochrome C), or endogenous biotin (e.g. liver, breast, brain, kidney) depending on the type of immunostain used. To differentiate endogenous enzyme activity or non-specific binding of enzymes from specific immunoreactivity, additional patient tissues may be stained exclusively with substrate chromogen or enzyme complexes (avidin-biotin, streptavidin, labelled polymer) and substrate-chromogen, respectively. If specific staining occurs in the negative tissue control, results with the patient specimens should be considered invalid.

Negative Reagent Control

Use a non-specific negative reagent control in place of the primary antibody with a section of each patient specimen to evaluate non-specific staining and allow better interpretation of specific staining at the antigen site.

Patient Tissue

Examine patient specimens stained with ORG-8936 last. Positive staining intensity should be assessed within the context of any non-specific background staining of the negative reagent control. As with any immunohistochemical test, a negative result means that the antigen was not detected, not that the antigen was absent in the cells/tissue assayed. If necessary, use a panel of antibodies to identify false-negative reactions.

Results Expected

Normal Tissues

Clone 1A5 was tested on a range of normal tissues (n=105). It detected the CD8 antigen on the cell surface of the cytotoxic sub-population of T cells in normal and inflamed tissues (18/18 tonsil, 1/1 skin; n=64/105 in total).

Tumor Tissues

Clone 1A5 stained 5/8 T cell lymphomas and infiltrating T cells in a variety of neoplastic tissues (n=80).

Intended for use on Ventana® Medical Systems Automated Immunohistochemistry Staining Systems.

General Limitations

Immunohistochemistry is a multistep diagnostic process that consists of specialized training in the selection of the appropriate reagents; tissue selection, fixation, and processing; preparation of the IHC slide; and interpretation of the staining results.

Tissue staining is dependent on the handling and processing of the tissue prior to staining. Improper fixation, freezing, thawing, washing, drying, heating, sectioning or contamination with other tissues or fluids may produce artefacts, antibody trapping, or false negative results. Inconsistent results may be due to variations in fixation and embedding methods, or to inherent irregularities within the tissue.[5]

Excessive or incomplete counterstaining may compromise proper interpretation of results.

The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

Origin™ Antibodies from Leica Biosystems Newcastle Ltd are for use, on paraffin-embedded sections with specific fixation requirements. Unexpected antigen expression may occur, especially in neoplasms. The clinical interpretation of any stained tissue section must include morphological analysis and the evaluation of appropriate controls

Bibliography – General

1. Clinical Laboratory Improvement Amendments of 1998: Final Rule 57 FR 7163. February, 1992.
2. National Committee for Clinical Laboratory Standards (NCCLS). Protection of laboratory workers from infectious diseases transmitted by blood and tissue; proposed guideline. Villanova, P.A. 1991; 7(9). Order code M29-P.
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4. Nadji M, Morales AR. Immunoperoxidase, part I: the techniques and pitfalls. Laboratory Medicine. 1983; 14:767.
5. Leong FJW-M and Leong AS-Y. Essential markers in malignant lymphoma: a diagnostic approach. The Journal of Histotechnology. 2002; 25(4):215–227.
6. 7. Middel P, Thelen P, Blaschke S et al. Expression of the T-cell chemoattractant chemokine lymphotactin in Crohn's disease. American Journal of Pathology. 2001; 159(5):1751–1761.

Trademarks

Explanation of Symbols

	Attention, see instructions for use		Temperature limitations	REF	Catalog number
IVD	In vitro diagnostic device	LOT	Batch number		
	Consult instructions for use		Use by		

Amendments to Previous Issue

Not applicable.

Date of Issue

31 July 2013

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