CE

FLEX
Monoclonal Mouse
Anti-Human CD1a
Clone 010
Ready-to-Use
(Link)

Code IR069

Intended use

For in vitro diagnostic use.

FLEX Monoclonal Mouse Anti-Human CD1a, Clone 010, Ready-to-Use (Link), is intended for use in immunohistochemistry (IHC) together with Autostainer Link instruments. The antibody labels CD1a-expressingcells in normal and neoplastic tissues. Results aid in the classification of thymomas and malignancies of T-cell precursors and Langerhans' cell histiocytosis (1). Differential classification is aided by the results from a panel of antibodies. 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. This antibody is intended to be used after the primary diagnosis of tumor has been made by conventional histopathology using nonimmunologic histochemical stains.

Summary and explanation

CD1a, a member of the CD1 antigen family, is a non-polymorphic MHC (major histocompatibility complex) class I-related cell surface glycoprotein expressed in association with ß$_2$-microglobulin (1, 2).

Refer to *Dako General Instructions for Immunohistochemical Staining* or the detection system instructions of IHC procedures for: Principle of Procedure, Materials Required, Not Supplied, Storage, Specimen Preparation, Staining Procedure, Quality Control, Troubleshooting, Interpretation of Staining, General Limitations.

Reagent provided

Ready-to-use monoclonal mouse antibody provided in liquid form in a buffer containing stabilizing protein and 0.015 mol/L sodium azide.

Clone: 010 (1). Isotype: IgG1, kappa.

Immunogen

Human thymus cells.

Specificity

Anti-Human CD1a, clone 010, was clustered as anti-CD1a at the 4th International Workshop and Conference on Human Leucocyte Differentiation Antigens (2). Clone 010 has been shown to immunoprecipitate labeled CD1a from thymus cell lysate and from Langerhans' cell-enriched epidermal cell lysate (2). In the latter, the antibody strongly precipitated the native CD1a molecule (49 kDa molecular weight) and ß$_2$-microglobulin (12 kDa molecular weight) but not the trypsin-cleaved CD1a molecule (27 kDa molecular weight) (2). In immunohistochemistry, the antibody has been demonstrated to react with the CD1a cell surface glycoprotein expressed on cortical thymocytes, Langerhans' cells, and interdigitating dendritic (reticulum) cells in frozen and formalin-fixed, paraffin-embedded (FFPE) tissues (1, 2).

Precautions

1. For in vitro diagnostic use.
2. For professional users.
3. This product contains sodium azide (NaN_3), a chemical highly toxic in pure form. At product concentrations, though not classified as hazardous, sodium azide may react with lead and copper plumbing to form highly explosive build-ups of metal azides. Upon disposal, flush with large volumes of water to prevent metal azide build-up in plumbing.
4. As with any product derived from biological sources, proper handling procedures should be used.
5. Wear appropriate Personal Protective Equipment to avoid contact with eyes and skin.
6. Unused solution should be disposed of according to local, State and Federal regulations.

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. 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 Support.

Specimen preparation

The antibody can be used for labeling formalin-fixed, paraffin-embedded tissue sections. Tissue specimens should be cut into sections of approximately 4 µm.

Pre-treatment with heat-induced epitope retrieval (HIER) is required. Optimal results are obtained by pretreating tissues using EnVision FLEX Target Retrieval Solution, High pH (50x) (Code K8004).

Deparaffinized sections: Pre-treatment of deparaffinized formalin-fixed, paraffin-embedded tissue sections is recommended using Dako PT Link. For details, please refer to the PT Link User Guide.

The following parameters should be used for PT Link: Pre-heat temperature: 65 °C; epitope retrieval temperature and time: 97 °C for 20 (±1) minutes; cool down to 65 °C. Remove Autostainer slide rack with slides from the PT

Link tank and immediately dip slides into a jar/tank (e.g., PT Link Rinse Station, Code PT109) containing diluted room temperature EnVision FLEX Wash Buffer (20x) (Code K8007). Leave slides in Wash Buffer for 1-5 minutes.

Paraffin-embedded sections: As alternative specimen preparation, both deparaffinization and epitope retrieval can be performed in the PT Link using a modified procedure. See the PT Link User Guide for instructions. After the staining procedure has been completed, the sections must be dehydrated, cleared and mounted using a permanent mounting method.

The tissue sections should not dry out during the treatment or during the following immunohistochemical staining procedure. For greater adherence of tissue sections to glass slides, the use of Dako Silanized Slides (Code S3003) is recommended.

Staining procedure

The recommended visualization system is EnVision FLEX+, Mouse, High pH (Link) (Code K8002). The staining steps and incubation times are pre-programmed into the Autostainer Link software. Please refer to the proper Autostainer Link User Guide for detailed instructions on loading slides and reagents. If the protocols are not available on the used Autostainer platform, please contact Dako Technical Support. All incubation steps should be performed at room temperature.

Optimal conditions may vary depending on specimen and preparation methods, and should be determined by each individual laboratory. Counterstaining in hematoxylin is recommended using EnVision FLEX Hematoxylin (Link) (Code K8008).

Positive and negative control tissues as well as negative control reagent should be run simultaneously using the same protocol as the patient specimens. The positive control tissue should include tonsil and skin and the cells/structures should display reaction patterns as described for this tissue in "Performance characteristics". The recommended negative control reagent is FLEX Negative Control, Mouse (Link) (Code IR750).

Staining interpretation

The cellular staining pattern is cytoplasmic and/or membranous (1, 2).

Performance characteristics

Normal tissues:

Anti-CD1a, clone 010 has been shown to label Langerhans' cells and variably to label interdigitating dendritic cells when tested on FFPE tonsil, and with Langerhans' cells when tested on paraffin-embedded skin (1). Any staining in germinal center of tonsils should be disregarded. Scattered labeling has been observed in interdigitating dendritic cells and medullary thymocytes in thymic medulla (1, 2). It should be noted that staining of smooth muscle cells may be observed.

Tissue Type (# tested)	Labeled Tissue Elements	Tissue Type (# tested)	Labeled Tissue Elements
Adrenal (3)	0/3	Pancreas (3)	2/3
Bone marrow (3)	0/3	Parathyroid (3)	0/3
Breast (3)	1/3	Pituitary (3)	0/3
Cerebellum (3)	0/3	Prostate (3)	0/3
Cerebrum (3)	0/3	Salivary gland (3)	0/3
Cervix (3)	1/3	Skeletal Muscle (3)	0/3
Colon (3)	0/3	Skin (3)	3/3
Esophagus (3)	3/3	Small intestine (3)	0/3
Heart (3)	0/3	Spleen (3)	0/3
Kidney (3)	0/3	Stomach (3)	0/3
Liver (3)	0/3	Testis (3)	0/3
Lung (3)	2/3	Thymus (3)	2/3
Mesothelial cells (3)	0/3	Thyroid (3)	0/3
Nerve, peripheral (3)	0/3	Tonsil (3)	3/3
Ovary (3)	1/3	Uterus (3)	0/3

Abnormal tissues:

Anti-CD1a, clone 010 has demonstrated the following results on abnormal paraffin-embedded tissues (1, 3, 4).

Tissue Type (# tested)	Labeled Tissue Elements
Lymph node: Paracortical hyperplasia (4)	3/4 variably-labeled, normal interdigitating dendritic cells
Lymph node: Dermatopathic lymphadenopathy (7)	7/7 labeled, normal large dendritic cells and veiled cells variably-labeled, normal interdigitating dendritic cells
Skin: Lymphoid hyperplasia (2)	2/2 labeled, normal Langerhans' cells and dermal dendritic cells variably labeled, normal interdigitating dendritic cells
Skin: Xanthoma (1)	1/1 labeled, normal Langerhans' cells
Cutaneous lymphoma: Mycosis fungoides (2)	2/2 labeled, normal Langerhans' cells, large dendritic cells, and dermal dendritic cells

Cutaneous lymphoma: Low-grade B-cell (1)	1/1 labeled, normal Langerhans' cells
Cutaneous lymphoma: T-immunoblastic (1)	1/1 labeled, normal Langerhans' cells, large dendritic cells, and dermal dendritic cells
Cutaneous lymphoma: Anaplastic (Ki-1+) large cell (1)	1/1 labeled, normal Langerhans' cells
Peripheral T-cell lymphoma (5)	0/5
Thymus: Hodgkin's disease (2)	2/2 labeled, normal cortical thymocytes and large dendritic cells variably labeled, normal interdigitating dendritic cells
Thymus: Thymoma of cortical type (2)	2/2 labeled, neoplastic cortical thymocytes
Maxillary Burkitt's lymphoma (1)	1/1 labeled, normal Langerhans' cells

[a]All skin specimens contained normal intraepidermal Langerhans' cells that were stained with Anti-CD1a, clone 010 (4).

References

1. Krenács L, Tiszalvicz L, Krenács T, Boumsell L. Immunohistochemical detection of CD1a antigen in formalin-fixed and paraffin-embedded tissue sections with monoclonal antibody 010. J Pathol 1993; 171:99–104.
2. Boumsell L. Cluster Report: CD1 in *Leucocyte Typing IV*. Eds. W. Knapp, B. Dörken, W. R. Gilks, E. P. Rieber, R. E. Schmidt, H. Stein, and A. E. G. Dr. von dem Borne. Oxford: Oxford UP, 1989; 251–69.
3. Emile JF, Wechsler J, Brousse N, Boulland ML, Cologon R, Fraitag S, et al. Langerhans' cell histiocytosis: Definitive diagnosis with the use of monoclonal antibody 010 on routinely paraffin-embedded samples. Amer J Surg Pathol 1995; 19:636–41.
4. Mazal PR, Hainfellner JA, Preiser J, Czech T, Simonitsch I, Radaszkiewicz T, Budka H. Langerhans' cell histiocytosis of the hypothalamus: Diagnostic value of immunohistochemistry. Clin Neuropath 1996; 15:87–91.

Explanation of symbols

REF	Catalogue number		Temperature limitation	IVD	In vitro diagnostic medical device
	Manufacturer	LOT	Batch code	Σ	Contains sufficient for <n> tests
	Use by		Consult instructions for use	EC REP	Authorized representative in the European Community

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