

CONFIRM anti-CD99 (O13) Mouse Monoclonal Primary Antibody

REF 790-4452

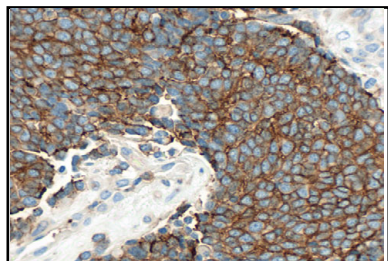


Figure 1. Ewing's sarcoma stained with CONFIRM anti-CD99 (O13) Mouse Monoclonal Antibody and the *ultraView* Universal DAB Detection Kit

INTENDED USE

Ventana Medical Systems' (Ventana) CONFIRM anti-CD99 (O13) Mouse Monoclonal Primary Antibody (CONFIRM anti-CD99 (O13)) is directed against human CD99 expressed on T lymphocytes, cortical thymocytes, granulosa cells of the ovary, pancreatic islet cells, CNS ependymal cells and Sertoli's cells. CD99 shows a plasma membrane location. This antibody may be used as part of a panel to aid in the identification of Ewing's sarcoma and related peripheral neuroectodermal tumors. The antibody is intended for

qualitative staining in sections of formalin fixed, paraffin embedded tissue. This product should be interpreted by a qualified pathologist in conjunction with histological examination, relevant clinical information, and proper controls.

This antibody is intended for *in vitro* diagnostic (IVD) use.

SUMMARY AND EXPLANATION

This mouse monoclonal antibody, also known as MIC-2, is a transmembrane glycoprotein present on the cell membrane of Ewing's sarcoma and primitive peripheral neuroectodermal tumors (PNET).¹ Within the hematopoietic system, CD99 has been implicated in cell adhesion and cell death, therefore contributing to the differentiation of T-cell precursors.² CD99 is widely distributed on many types of normal cells, with a particularly strong expression on cells of the T-cell lineage. Expression is seen in normal cells such as pancreatic islet cells, cortical thymocytes, Sertoli cells and ovarian granulosa cells.¹ Mature granulocytes express very little or no CD99. Immunoreactivity to the protein has also been observed in a broad range of neoplastic tissues, including gastrointestinal and pulmonary neuroendocrine tumors, sex cord-stromal tumors, lymphoblastic lymphoma, and a small percentage of breast carcinomas.³⁻⁶

REAGENT PROVIDED

CONFIRM anti-CD99 (O13) Mouse Monoclonal Primary Antibody contains sufficient reagent for staining 50 tests.

One 5 mL dispenser of CONFIRM anti-CD99 (O13) contains approximately 2.65 µg of a mouse monoclonal antibody.

The antibody is diluted in a phosphate buffered saline containing carrier protein and a preservative.

Total protein concentration of the reagent is approximately 3 mg/mL. Specific antibody concentration is approximately 0.531 µg/mL. There is no known non specific antibody reactivity observed in this product.

CONFIRM anti-CD99 (O13) is a monoclonal antibody produced from mouse ascites.

Refer to the appropriate Ventana detection kit package insert for detailed descriptions of:

- (1) Principles and Procedures, (2) Materials and Reagents Needed but Not Provided, (3) Specimen Collection and Preparation for Analysis, (4) Quality Control Procedures, (5) Troubleshooting, (6) Interpretation of Results, and (7) General Limitations.

MATERIALS REQUIRED BUT NOT PROVIDED

Staining reagents such as Ventana detection kits (*ultraView* Universal DAB Detection Kits), and ancillary components, including negative and positive tissue control slides, are not provided.

STORAGE

Store at 2-8°C. Do not freeze.

To ensure proper reagent delivery and the stability of the antibody, replace the dispenser cap after every use and immediately place the dispenser in the refrigerator in an upright position.

Every antibody dispenser is expiration dated. When properly stored, the reagent is stable to the date indicated on the label. Do not use reagent beyond the expiration date.

SPECIMEN PREPARATION

Routinely processed, formalin fixed, paraffin embedded tissues are suitable for use with this primary antibody when used with Ventana detection kits and a Ventana automated slide stainer. The recommended tissue fixative is 10% neutral buffered formalin.⁷ Slides should be stained immediately, as antigenicity of cut tissue sections may diminish over time.

It is recommended that positive and negative controls be run simultaneously with unknown specimens.

WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic use.
2. This product contains 1% or less bovine serum which is used in the manufacture of the antibody.
3. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
4. Avoid microbial contamination of reagents.
5. Consult local and/or state authorities with regard to recommended method of disposal.

STAINING PROCEDURE

Ventana primary antibodies have been developed for use on a Ventana automated slide stainer in combination with Ventana detection kits and accessories. A recommended staining protocol for the BenchMark XT/BenchMark ULTRA instrument with *ultraView* Universal DAB Detection Kit is listed in Table 1.

The parameters for the automated procedures can be displayed, printed and edited according to the procedure in the instrument's Operator's Manual. Refer to the appropriate Ventana detection kit package insert for more details regarding immunohistochemistry staining procedures.

Table 1. Recommended Staining Protocol for CONFIRM anti-CD99 (O13) with *ultraView* Universal DAB Detection Kit on a BenchMark XT/BenchMark ULTRA instrument

Procedure Type	Method
Deparaffinization	Selected
Cell Conditioning (Antigen Unmasking)	Cell Conditioning 1 Standard
Enzyme (Protease)	None Required
Antibody (Primary)	BenchMark XT instrument Approximately 16 Minutes, 37°C BenchMark ULTRA instrument Approximately 16 minutes, 36°C
Counterstain	Hematoxylin II, 4 minutes
Post Counterstain	Bluing Reagent, 4 minutes

Due to variation in tissue fixation and processing, as well as general lab instrument and environmental conditions, it may be necessary to increase or decrease the primary antibody incubation, cell conditioning or protease pretreatment based on individual specimens, detection used, and reader preference. For further information on fixation variables, refer to "Immunohistochemistry Principles and Advances".⁸

POSITIVE TISSUE CONTROL

Examples of positive control tissues for this antibody are pancreatic islet cells and Sertoli cells of the testis.

STAINING INTERPRETATION

The cellular staining pattern for CONFIRM anti-CD99 (O13) is membranous.

SPECIFIC LIMITATIONS

This antibody has been optimized for a 16 minute incubation time on a BenchMark XT/BenchMark ULTRA instrument in combination with *ultraView* Universal DAB Detection Kit (REF 760-500) but the user must validate results obtained with this reagent.

PERFORMANCE CHARACTERISTICS

1. Specificity of CONFIRM anti-CD99 (O13) was determined by testing formalin fixed, paraffin embedded normal and neoplastic tissues.

For normal tissues, results are as follows: (0/3) cerebrum, (0/3) cerebellum, (0/3) adrenal gland, (0/3) ovary, (3/6) pancreas, (0/3) parathyroid, (0/3) hypophysis, (7/7) testis, (0/3) thyroid gland, (0/3) breast, (1/3) spleen, (0/3) tonsil, (3/3) thymus, (0/3) myeloid, (0/3) lung, (0/3) esophagus, (0/3) stomach, (0/3) small intestine, (0/3) colon, (0/3) liver, (0/3) salivary gland, (2/3) kidney, (2/3) prostate, (0/3) endometrium, (0/3) cervix, (0/3) skeletal muscle, (0/3) skin, (0/3) mesothelium and lung.

For neoplastic tissues, results are as follows: (1/1) glioblastoma, (0/1) atypical meningioma, (0/1) malignant ependymoma, (0/1) oligodendroglioma, (0/1) serous papillary adenocarcinoma, (0/1) mucinous papillary adenocarcinoma, (3/9) islet cell carcinoma, (0/1) pancreatic adenocarcinoma, (0/1) seminoma, (0/1) embryonal carcinoma, (0/1) medullary carcinoma, (0/1) papillary carcinoma, (0/1) intraductal carcinoma, (0/1) lobular breast carcinoma in situ, (0/1) invasive ductal carcinoma, (0/1) diffuse B-cell lymphoma, (0/1) small cell undifferentiated carcinoma, (0/1) lung squamous cell carcinoma, (0/1) lung adenocarcinoma, (0/1) esophagus squamous cell carcinoma, (0/1) esophagus adenocarcinoma, (0/1) stomach mucinous adenocarcinoma, (0/1) small intestine adenocarcinoma, (0/3) GIST, (0/1) colon adenocarcinoma, (0/1) rectal adenocarcinoma, (0/1) hepatocellular carcinoma, (1/1) hepatoblastoma, (0/1) clear cell carcinoma, (0/1) prostate adenocarcinoma, (0/1) transitional cell prostate carcinoma, (0/1) leiomyoma, (0/1) endometrial adenocarcinoma, (0/1) clear cell carcinoma of endometrium, (0/7) embryonal rhabdomyosarcoma, (0/1) malignant melanoma, (0/1) basal cell carcinoma, (0/3) squamous cell carcinoma, (1/1) neurofibroma, (0/1) neuroblastoma, (1/1) epithelial malignant mesothelioma, (1/3) diffuse malignant lymphoma, (0/1) Hodgkin's lymphoma, (0/1) transitional cell carcinoma with squamous metaplasia, (0/9) leiomyosarcoma, (0/1) osteosarcoma, (0/1) spindle cell rhabdomyosarcoma, (0/4) endodermal tumor, (3/4) embryonal carcinoma, (4/5) PNET, (0/20) adrenal cortical adenocarcinoma, (0/10) neuroendocrine carcinoma, (0/17) carcinoid, (3/22) atypical carcinoid, (0/1) chronic inflammation of hyperplastic fibrous tissue, (1/7) malignant fibrous histiocytoma, (0/6) synovial sarcoma, (0/3) liposarcoma, (0/2) extracellular osteosarcoma, (0/1) malignant peripheral nerve sheath tumor, (0/2) chordoma, (0/1) malignant mesenchymoma metastatic.

2. Inter-lot reproducibility was determined by testing 3 lots across 1 multi-tissue block (3 tissues per block, 2 slides per lot) on a BenchMark XT instrument. 18 out of 18 tested across all 3 lots scored equivalently.
3. Inter-run repeatability was determined by staining 2 multi-tissue blocks (3 tissues per block for a total of 6 tissues) across 5 slides on a BenchMark XT instrument over a 5 day non-consecutive period. 150 out of 150 samples tested scored equivalently.
4. Intra-run repeatability was determined by staining 2 multi-tissue blocks (3 tissues per block for a total of 6 tissues) across 14 slides on a BenchMark XT instrument. 84 out of 84 samples tested scored equivalently.
5. Intra-platform repeatability was determined by staining 2 multi-tissue blocks (3 tissues per block for a total of 6 tissues) across 5 slides on 3 BenchMark XT instruments. 90 out of 90 samples tested scored equivalently.
6. Intra-platform repeatability was determined by staining 1 multi-tissue block (3 tissues per block) across 5 slides on 3 BenchMark ULTRA instruments. 45 out of 45 samples tested scored equivalently.
7. Inter-platform repeatability was determined by staining 1 multi-tissue block (3 tissues per block) across 5 slides on 3 BenchMark XT instruments and 3 BenchMark ULTRA instruments. 90 out of 90 samples tested scored equivalently.
8. Compatible with MIEW DAB and *ultraView* Universal DAB Detection Kits.

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