CONFIRM anti-MITF (C5/D5) Mouse Monoclonal Primary Antibody

REF 790-4367

05549175001

IVD Σ 50

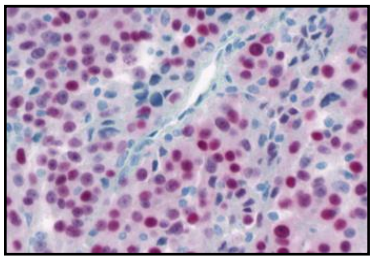

Figure 1. CONFIRM anti-MITF (C5/D5) antibody nuclear staining of melanoma.

INTENDED USE

CONFIRM anti-MITF (C5/D5) Mouse Monoclonal Primary Antibody is a mouse monoclonal antibody cocktail intended for laboratory use in the qualitative immunohistochemical detection of microphthalmia-associated transcription factor (MITF) protein by light microscopy in sections of formalin-fixed, paraffin-embedded tissue on a BenchMark IHC/ISH instrument. 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

CONFIRM anti-MITF (C5/D5) Mouse Monoclonal Primary Antibody (CONFIRM anti-MITF (C5/D5) antibody) is a mouse monoclonal antibody cocktail produced against MITF proteins in normal and neoplastic tissues. MITF is a basic helix-loop-helix-leucine-zipper transcription factor which regulates the development and survival of melanocytes and retinal pigment epithelium, and is also involved in transcription of melanin epithelioid pigmentation enzyme genes such as tyrosinase.[1,2,3] Multiple isoforms of MITF exist including MITF-A, MITF-B, MITF-C, MITF-H, and MITF-M, which differ in the amino terminal domain and in their expression patterns. The MITF-M isoform is restricted to the melanocyte cell lineage and is typically expressed in neoplastic cells.[3]

Detection of MITF protein by immunohistochemistry (IHC) with the CONFIRM anti-MITF (C5/D5) antibody may be used as a melanocyte marker to aid in the identification of melanocytic differentiation in benign and malignant lesions. It may be used as part of a panel of IHC studies. The staining pattern is nuclear.

PRINCIPLE OF THE PROCEDURE

The CONFIRM anti-MITF (C5/D5) antibody binds to MITF proteins in formalin-fixed, paraffin-embedded (FFPE) tissue sections. This antibody can be visualized using *ultra*View Universal Alkaline Phosphatase Red Detection Kit (Cat. No. 760-501 / 05269814001). Refer to the *ultra*View Universal Alkaline Phosphatase Red Detection Kit method sheet for further information.

In addition to staining with the CONFIRM anti-MITF (C5/D5) antibody, a second slide should be stained with the appropriate negative control reagent.

MATERIAL PROVIDED

CONFIRM anti-MITF (C5/D5) antibody contains sufficient reagent for staining 50 slides.

One 5 mL dispenser of CONFIRM anti-MITF (C5/D5) antibody contains approximately 2.8 µg of the C5 clone and 33.8 µg of the D5 clone.

The antibody is diluted in 0.05 M Tris-HCL with 7% carrier protein and 0.10% ProClin 300, a preservative.

Total protein concentration of the reagent is approximately 10 mg/mL. Specific antibody concentration is approximately 0.6 µg/mL of the C5 clone and approximately 6.8 µg/mL of the D5 clone.

CONFIRM anti-MITF (C5/D5) antibody is a mouse monoclonal antibody cocktail produced as unpurified cell culture supernatant.

Refer to the appropriate VENTANA detection kit method sheet for detailed descriptions of: Principle of the Procedure, Material and Methods, Specimen Collection and Preparation for Analysis, Quality Control Procedures, Troubleshooting, Interpretation of Results, and General Limitations.

MATERIALS REQUIRED BUT NOT PROVIDED

Staining reagents, such as VENTANA detection kits and ancillary components, including negative and positive tissue control slides, are not provided.

Not all products listed in the method sheet may be available in all geographies. Consult your local support representative.

The following reagents and materials may be required for staining but are not provided:

1. Recommended control tissue
2. Microscope slides, positively charged
3. *ultra*View Universal Alkaline Phosphatase Red Detection Kit (Cat. No. 760-501 / 05269814001)
4. Negative Control (Monoclonal) (Cat. No. 760-2014 / 05266670001)
5. EZ Prep Concentrate (10X) (Cat. No. 950-102 / 05279771001)
6. Reaction Buffer Concentrate (10X) (Cat. No. 950-300 / 05353955001)
7. LCS (Predilute) (Cat. No. 650-010 / 05264839001)
8. ULTRA LCS (Predilute) (Cat. No. 650-210 / 05424534001)
9. Cell Conditioning Solution (CC1) (Cat. No. 950-124 / 05279801001)
10. ULTRA Cell Conditioning Solution (ULTRA CC1) (Cat. No. 950-224 / 05424569001)
11. Antibody Diluent (Cat. No. 251-018 / 15261899001)
12. Hematoxylin II (Cat. No. 790-2208 / 05277965001)
13. Bluing Reagent (Cat. No. 760-2037 / 05266769001)
14. Permanent mounting medium
15. Cover glass
16. Automated coverslipper
17. General purpose laboratory equipment
18. BenchMark IHC/ISH instrument

STORAGE AND STABILITY

Upon receipt and when not in use, 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 FFPE tissues are suitable for use with this primary antibody when used with VENTANA detection kits and BenchMark IHC/ISH instruments. The recommended tissue fixative is 10% neutral buffered formalin.[4] 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 (IVD) use.
2. For professional use only.
3. **CAUTION:** In the United States, Federal law restricts this device to sale by or on the order of a physician. (Rx Only)
4. Do not use beyond the specified number of tests.
5. ProClin 300 solution is used as a preservative in this reagent. It is classified as an irritant and may cause sensitization through skin contact. Take reasonable precautions when handling. Avoid contact of reagents with eyes, skin, and mucous membranes. Use protective clothing and gloves.
6. This product contains 2% or less bovine serum which is used in the manufacture of the antibody.
7. Positively charged slides may be susceptible to environmental stresses resulting in inappropriate staining of any IHC assay (for example, lack of primary antibody or counterstain on the tissue). Ask your Roche representative for a copy of “Impacts of Environmental Stresses on IHC Positively Charged Slides” to better understand how to use these types of slides.

8. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions. In the event of exposure, the health directives of the responsible authorities should be followed.[5,6]
9. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
10. Avoid microbial contamination of reagents as it may cause incorrect results.
11. For further information on the use of this device, refer to the BenchMark IHC/ISH instrument Operator's Manual, and instructions for use of all necessary components.
12. Consult local and/or state authorities with regard to recommended method of disposal.
13. Product safety labeling primarily follows EU GHS guidance. Safety data sheet available for professional user on request.
14. To report suspected serious incidents related to this device, contact the local Roche representative and the competent authority of the Member State or Country in which the user is established.

This product contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Table 1. Hazard information.

Hazard	Code	Statement
 Warning	H317	May cause an allergic skin reaction.
	H412	Harmful to aquatic life with long lasting effects.
	P261	Avoid breathing dust/fume/gas/mist/vapours/spray.
	P272	Contaminated work clothing should not be allowed out of the workplace.
	P280	Wear protective gloves.
	P333 + P313	If skin irritation or rash occurs: Get medical advice/ attention.
	P362 + P364	Take off contaminated clothing and wash it before reuse.
	P501	Dispose of contents/ container to an approved waste disposal plant.

This product contains CAS # 55965-84-9, a mixture of: 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-2H-isothiazol-3-one (3:1).

STAINING PROCEDURE

VENTANA primary antibodies have been developed for use on BenchMark IHC/ISH instruments in combination with VENTANA detection kits and accessories. Refer to Table 2 for recommended staining protocols.

This antibody has been optimized for specific incubation times but the user must validate results obtained with this reagent.

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

For more details on the proper use of this device, refer to the inline dispenser method sheet associated with P/N 790-4367.

Table 2. Recommended staining protocol for CONFIRM anti-MITF (C5/D5) antibody with *ultra*View Universal Alkaline Phosphatase Red Detection Kit on BenchMark IHC/ISH instruments.

Procedure Type	Method	
	XT	ULTRA
Deparaffinization	Selected	Selected
Cell Conditioning (Antigen Unmasking)	CC1, Mild	ULTRA CC1 36 minutes, 95°C
Antibody (Primary)	16 minutes, 37°C	16 minutes, 36°C
ultraBlock (Required)	8 minutes	
Counterstain	Hematoxylin II, 4 minutes	
Post Counterstain	Bluing, 4 minutes	

*Use of Antibody Diluent at the ultraBlock step is required.

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."[7]

POSITIVE TISSUE CONTROL

Optimal laboratory practice is to include a positive control section on the same slide as the test tissue. This helps identify any failures applying reagents to the slide. Tissue with weak positive staining is best suited for quality control. Control tissue may contain both positive and negative staining elements and serve as both the positive and negative control.

Control tissue should be fresh autopsy, biopsy, or surgical specimen, prepared or fixed as soon as possible in a manner identical to test sections.

Known positive tissue controls should be utilized only for monitoring performance of reagents and instruments, not as an aid in determining specific diagnosis of test samples. If the positive tissue controls fail to demonstrate positive staining, results of the test specimen should be considered invalid.

Examples of positive control tissues for CONFIRM anti-MITF (C5/D5) antibody are primary melanoma tumor cells as depicted in Figure 1 and melanocytes in normal skin.

STAINING INTERPRETATION / EXPECTED RESULTS

The cellular staining pattern for CONFIRM anti-MITF (C5/D5) antibody is nuclear.

SPECIFIC LIMITATIONS

Necrosis and macrophages are often present in melanoma and may stain non-specifically. Due to this, a blocking reagent must be used in conjunction with this product.

Only tumor cells exhibiting a nuclear staining pattern should be interpreted.

All assays might not be registered on every instrument. Please contact your local Roche representative for more information.

PERFORMANCE CHARACTERISTICS

ANALYTICAL PERFORMANCE

Staining tests for sensitivity, specificity, and precision were conducted and the results are listed below.

Sensitivity and Specificity

Table 3. Sensitivity/Specificity of CONFIRM anti-MITF (C5/D5) antibody was determined by testing normal FFPE tissues.

Tissue	# positive / total cases	Tissue	# positive / total cases
Cerebrum	0/3	Thymus	0/3
Cerebellum	0/3	Myeloid (bone marrow)	0/3
Adrenal gland	0/3	Lung	0/3
Ovary	0/3	Heart	0/3
Pancreas	0/3	Esophagus	0/3
Parathyroid gland	0/3	Stomach	0/3
Hypophysis (Pituitary)	0/3	Small intestine	0/3
Testis	0/3	Colon	0/3

Tissue	# positive / total cases	Tissue	# positive / total cases
Thyroid	0/3	Liver	0/3
Breast	0/3	Salivary gland	0/3
Spleen	0/3	Kidney	0/3
Tonsil	0/3	Prostate	0/3
Endometrium	0/3	Cervix	0/2
Skeletal muscle	0/3	Skin	2/2
Nerve	0/3	Mesothelium	0/3

Table 4. Sensitivity/Specificity of CONFIRM anti-MITF (C5/D5) antibody was determined by testing a variety of FFPE neoplastic tissues.

Pathology	# positive / total cases
Glioblastoma (Cerebrum)	0/1
Meningioma (Cerebrum)	0/1
Ependymoma (Cerebrum)	0/1
Oligodendroglioma (Cerebrum)	0/1
Serous carcinoma (Ovary)	0/1
Mucinous carcinoma (Ovary)	0/1
Pancreatic neuroendocrine neoplasm (Pancreas)	0/1
Adenocarcinoma (Pancreas)	0/1
Seminoma (Testis)	0/1
Embryonal carcinoma (Testis)	0/1
Medullary carcinoma (Thyroid)	0/1
Papillary carcinoma (Thyroid)	0/1
Ductal carcinoma in situ (Breast)	0/1
Invasive lobular carcinoma (Breast)	0/1
Invasive ductal carcinoma (Breast)	0/1
B-cell Lymphoma; NOS (Spleen)	0/1
Small cell carcinoma (Lung)	0/1
Squamous cell carcinoma (Lung)	0/1
Adenocarcinoma (Lung)	0/1
Squamous cell carcinoma (Esophagus)	0/1
Adenocarcinoma (Esophagus)	0/1
Adenocarcinoma (Stomach)	0/1
Adenocarcinoma (Small Intestine)	0/1
Gastrointestinal stromal tumor (GIST) (Small Intestine)	0/1
Adenocarcinoma (Colon)	0/2
Gastrointestinal stromal tumor (GIST) (Colon)	0/1
Gastrointestinal stromal tumor (GIST) (Rectum)	0/1
Hepatocellular carcinoma (Liver)	0/1
Hepatoblastoma (Liver)	0/1
Clear cell carcinoma (Kidney)	0/1
Adenocarcinoma (Prostate)	0/1
Urothelial carcinoma (Bladder)	0/1
Urothelial carcinoma (Prostatic urethra)	0/1
Leiomyoma (Uterus)	0/1
Carcinoma (Endometrium)	0/1
Clear cell and squamous carcinomas (Uterus)	0/3
Embryonal rhabdomyosarcoma	0/1
Melanoma (Rectum)	0/1
Basal cell carcinoma (Skin)	0/1
Squamous cell carcinoma (Skin)	0/1
Neurofibroma (Lumbar)	0/1
Neuroblastoma	0/1
Mesothelioma	0/1
Hodgkin lymphoma (Lymph node)	0/1
Lymphoma; NOS	0/3
Leiomyosarcoma	0/2
Osteosarcoma	0/1
Spindle cell rhabdomyosarcoma	0/1
Melanoma	93/171
Melanoma (Metastatic)	9/17
Melanocytic nevus	18/22

Precision

Precision studies for CONFIRM anti-MITF (C5/D5) antibody were completed to demonstrate:

- Between lot precision of the antibody.
- Within run and between day precision on a BenchMark XT instrument.
- Between instrument precision on the BenchMark XT and BenchMark ULTRA instrument.
- Between platform precision between the BenchMark XT and BenchMark ULTRA instrument.

All studies met their acceptance criteria.

CLINICAL PERFORMANCE

Clinical performance data relevant to the intended purpose of CONFIRM anti-MITF (C5/D5) antibody were assessed by systematic review of the literature. The data gathered support the use of the device in accordance with its intended purpose.

REFERENCES

1. Tachibana M, Perez-Jurado LA, Nakayama A, et al. Cloning of MITF, the human homolog of the mouse microphthalmia gene and assignment to chromosome 3p14.1-p12.3. Hum Mol Genet. 1994; 3(4):553

2. Chang KL, Folpe AL. Diagnostic utility of microphthalmia transcription factor in malignant melanoma and other tumors. Adv Anat Pathol. 2001; 8(5):273
3. Widlund HR, Fisher DE. Microphthalamia-associated transcription factor: a critical regulator of pigment cell development and survival. Oncogene. 2003; 22(20):3035
4. Sheehan DC, Hrapchak BB. Theory and Practice of Histotechnology 2nd Edition. The C.V. Mosby Company, St. Louis, 1980
5. Occupational Safety and Health Standards: Occupational exposure to hazardous chemicals in laboratories. (29 CFR Part 1910.1450). Fed. Register.
6. Directive 2000/54/EC of the European Parliament and Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work.
7. Roche PC, Hsi ED. Immunohistochemistry-Principles and Advances. Manual of Clinical Laboratory Immunology, 6th edition. In: NR Rose, ed. ASM Press; 2002

NOTE: A point (period/stop) is always used in this document as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

The summary of safety and performance can be found here:

https://ec.europa.eu/tools/eudamed

Symbols

Ventana uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see dialog.roche.com for definition of symbols used):

GTIN Global Trade Item Number

INTELLECTUAL PROPERTY

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