CONFIRM anti-Total c-MET (SP44) Rabbit Monoclonal Primary Antibody

REF 790-4430

05571219001

IVD Σ 50

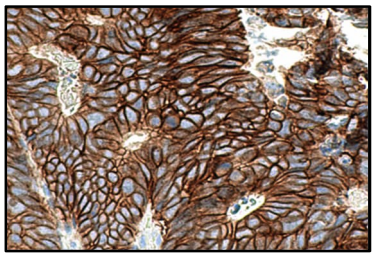

Figure 1. CONFIRM anti-Total c-MET (SP44) Rabbit Monoclonal Primary Antibody cytoplasmic membrane staining of colon carcinoma.

INTENDED USE

Ventana Medical Systems' (Ventana) CONFIRM anti-Total c-MET (SP44) Rabbit Monoclonal Primary Antibody is directed against a membranous and/or cytoplasmic epitope present in human normal epithelial or tumor cells. This antibody may be used to aid in the identification of normal and neoplastic c-MET expressing cells. The antibody is intended for qualitative staining in sections of formalin fixed, paraffin embedded tissue.

The clinical interpretation of any staining, or the absence of staining, must be complemented by histological studies and evaluation of proper controls. Evaluation must be made by a qualified pathologist within the context of the patient's clinical history and other diagnostic tests.

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

SUMMARY AND EXPLANATION

CONFIRM anti-Total c-MET (SP44) Rabbit Monoclonal Primary Antibody (CONFIRM anti-Total c-MET (SP44) antibody) is a rabbit monoclonal antibody against the carboxyl region of the transmembrane human c-MET protein. The c-MET protein is a receptor tyrosine kinase encoded by the c-MET proto-oncogene. Deregulated c-MET expression has been detected at both protein and mRNA levels in a variety of human carcinomas and sarcomas. CONFIRM anti-Total c-MET (SP44) antibody stains hepatomas, carcinomas of colon and rectum, stomach, kidney, ovary, skin, lung, thyroid and pancreas.[1] Tumors derived from c-MET expressing epithelia are usually positive; these include colorectal carcinomas, gastric adenocarcinomas, and non-small cell lung carcinomas.

In gastric cancer and non-small cell lung carcinoma, it has been determined that c-MET drives the cancer.[2,3] It has also been found that c-MET is a resistance pathway in lung cancer for EGFR inhibitors.[4]

PRINCIPLE OF THE PROCEDURE

CONFIRM anti-Total c-MET (SP44) antibody binds to the c-MET protein in formalin-fixed, paraffin-embedded (FFPE) tissue sections and exhibits membranous and/or cytoplasmic staining pattern. This antibody can be visualized using *ultra*View Universal DAB Detection Kit. Refer to the respective method sheet for further information.

MATERIAL PROVIDED

CONFIRM anti-Total c-MET (SP44) antibody contains sufficient reagent for 50 tests.

One 5 mL dispenser of CONFIRM anti-Total c-MET contains approximately 48.75 µg of a rabbit monoclonal (SP44) antibody.

The antibody is diluted in 0.05 M Tris-HCL with 1% carrier protein and 0.10% ProClin 300, a preservative.

Specific antibody concentration is approximately 9.75 µg/mL. There is no known non-specific antibody reactivity observed in this product.

CONFIRM anti-Total c-MET (SP44) antibody is a recombinant rabbit monoclonal antibody.

Refer to the appropriate VENTANA detection kit method sheet for detailed descriptions of: Principles and Procedures, Materials and Methods, Specimen Collection and Preparation for Analysis, Quality Control Procedures, Troubleshooting, Interpretation of Results, and 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. Rabbit Monoclonal Negative Control Ig (Cat. No. 790-4795 / 06683380001)
4. CONFIRM Negative Control Rabbit Ig (Cat. No. 760-1029 / 05266238001)
5. *ultra*View Universal DAB Detection Kit (Cat. No. 760-500 / 05269806001)
6. EZ Prep Concentrate (10X) (Cat. No. 950-102 / 05279771001)
7. Reaction Buffer Concentrate (10X) (Cat. No. 950-300 / 05353955001)
8. LCS (Predilute) (Cat. No. 650-010 / 05264839001)
9. ULTRA LCS (Predilute) (Cat. No. 650-210 / 05424534001)
10. Cell Conditioning Solution (CC1) (Cat. No. 950-124 / 05279801001)
11. ULTRA Cell Conditioning Solution (ULTRA CC1) (Cat. No. 950-224 / 05424569001)
12. Hematoxylin II (Cat. No. 790-2208 / 05277965001)
13. Bluing Reagent (Cat. No. 760-2037 / 05266769001)
14. General purpose laboratory equipment
15. BenchMark IHC/ISH instrument

STORAGE AND STABILITY

Upon receipt and when not in use, store at 2 to 8°C. Do not freeze.

To ensure proper reagent delivery and stability of the antibody, the cap must be replaced and the dispenser must be immediately placed in the refrigerator in an upright position after every use.

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.[5] Sections should be cut at approximately 4 µm in thickness and mounted on positively charged slides. 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. This product contains 1% or less bovine serum which is used in the manufacture of the antibody.
6. 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.
7. Positively charged slides may be susceptible to environmental stresses resulting in inappropriate staining. Ask your Roche representative for more information on 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.[6,7]
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.
11. For further information on the use of this device, refer to the BenchMark IHC/ISH instrument User Guide, and instructions for use of all necessary components located at navifyportal.roche.com.

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 mist or vapours.
	P273	Avoid release to the environment.
	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, reaction mass of: 5-chloro-2-methyl-2H-isothiazol-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. Recommended staining protocols for BenchMark XT and BenchMark ULTRA instruments with *ultra*View Universal DAB Detection Kit are listed in Table 2.

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 User Guide. Refer to the appropriate VENTANA detection 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 790-4430.

Table 2. Recommended Staining Protocols for CONFIRM anti-Total c-MET (SP44) antibody with *ultra*View Universal DAB Detection Kit on BenchMark IHC/ISH instruments.

Procedure Type	Method	
	XT	ULTRA
Deparaffinization	Selected	Selected
Cell Conditioning (Antigen Unmasking)	Standard Cell Conditioning 1	Standard Cell Conditioning 1
Enzyme (Protease)	None required	None required
Antibody (Primary)	Approximately 16 minutes, 37˚C	Approximately 16 minutes, 36˚C
Counterstain	Hematoxylin II, 4 minutes	Hematoxylin II, 4 minutes
Post Counterstain	Bluing Reagent, 4 minutes	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 and cell conditioning based on individual specimens, detection used, and reader preference. For further information on fixation variables, refer to "Immunohistochemistry Principles and Advances."[8]

NEGATIVE REAGENT CONTROL

In addition to staining with CONFIRM anti-Total c-MET (SP44) antibody, a second slide should be stained with the appropriate negative control reagent.

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 tissue control cell types and tissues for CONFIRM anti-Total c-MET (SP44) antibody are epithelial cells and neoplastic tissues such as gastric carcinoma and non-small cell lung carcinoma.

STAINING INTERPRETATION / EXPECTED RESULTS

The cellular staining pattern for CONFIRM anti-Total c-MET (SP44) antibody is membranous and/or cytoplasmic.

SPECIFIC LIMITATIONS

CONFIRM anti-Total c-MET (SP44) antibody has been optimized on BenchMark XT and BenchMark ULTRA instruments in combination with *ultra*View Universal DAB Detection Kit at a 16 minute primary antibody incubation time; however, the user must validate individual laboratory results obtained with this reagent.

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-Total c-MET (SP44) antibody was determined by testing FFPE normal tissues.

Tissue	# Positive / Total Cases	Tissue	# Positive / Total Cases
Cerebrum	0/3	Heart	0/3
Cerebellum	0/3	Esophagus	0/3
Adrenal gland	0/3	Stomach	0/3
Ovary	0/3	Small intestine	0/3
Pancreas	0/3	Colon	0/3
Parathyroid gland	0/3	Liver	0/3
Pituitary gland	0/3	Salivary gland	0/3
Testis	0/3	Kidney	0/3
Thyroid	0/3	Prostate	0/3
Breast	0/3	Endometrium	1/3
Spleen	0/3	Cervix	0/2
Tonsil	0/3	Skeletal muscle	0/3
Thymus	0/3	Skin	0/3
Myeloid (bone marrow)	0/3	Nerve	0/3

Tissue	# Positive / Total Cases	Tissue	# Positive / Total Cases
Lung	1/3	Mesothelium	0/3

Table 4. Sensitivity/Specificity of CONFIRM anti-Total c-MET (SP44) antibody was determined by testing a variety of FFPE neoplastic tissues.

Pathology	# Positive / Total Cases
Glioblastoma	0/1
Meningioma	0/1
Ependymoma	0/1
Oligodendroglioma	0/1
Serous adenocarcinoma (Ovary)	0/1
Mucinous adenocarcinoma (Ovary)	1/1
Neuroendocrine neoplasm (Pancreas)	0/1
Adenocarcinoma (Pancreas)	1/1
Seminoma	0/1
Embryonal carcinoma	0/1
Medullary carcinoma (Thyroid)	0/1
Papillary carcinoma (Thyroid)	1/1
Ductal carcinoma in situ (Breast)	0/1
Lobular carcinoma (Breast)	0/1
Invasive ductal carcinoma (Breast)	0/1
B-cell lymphoma, NOS	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)	1/1
Mucinous adenocarcinoma (Stomach)	0/1
Adenocarcinoma (Intestine)	0/1
Gastrointestinal stromal tumor (GIST) (Intestine)	0/1
Adenocarcinoma (Colon)	1/1
Gastrointestinal stromal tumor (GIST) (Colon)	0/1
Adenocarcinoma (Rectum)	0/1
Gastrointestinal stromal tumor (GIST) (Rectum)	0/1
Hepatocellular carcinoma	0/1
Hepatoblastoma	0/1
Clear cell carcinoma	1/1
Adenocarcinoma (Prostate)	0/1
Urothelial carcinoma (Prostate)	0/1
Leiomyoma	0/1
Adenocarcinoma (Endometrium)	0/1
Clear cell carcinoma (Endometrium)	0/1
Squamous cell carcinoma (Cervix)	0/2
Embryonal rhabdomyosarcoma	0/1
Melanoma (Skin)	0/1
Basal cell carcinoma (Skin)	0/1
Squamous cell carcinoma (Skin)	0/1
Neurofibroma	0/1
Neuroblastoma (Retroperitoneum)	0/1
Epithelioid mesothelioma	1/1
Lymphoma, NOS	0/3
Hodgkin lymphoma	0/1
Urothelial carcinoma (Bladder)	0/1
Leiomyosarcoma	0/2
Osteosarcoma (Bone)	0/1
Spindle cell rhabdomyosarcoma (Retroperitoneum)	0/1
Carcinoma, NOS (Breast)	2/63
Carcinoma, NOS (Colon)	73/98
Non-small cell carcinoma (Lung)	7/69
Carcinoma, NOS (Kidney)	12/24
Carcinoma, NOS (Ovary)	6/32
Carcinoma, NOS (Stomach)	19/75

Precision

Precision studies for CONFIRM anti-Total c-MET (SP44) 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, BenchMark ULTRA instrument.
- Between platform precision between the BenchMark XT, BenchMark ULTRA instrument.

All studies met their acceptance criteria.

REFERENCES

1. Prat M, Narsimhan RP, et al. The Receptor Encoded by the Human c-MET Oncogene is Expressed in Hepatocytes, Epithelial cells and solid tumors. Int. J. Cancer: 1991;49, 323-328.
2. Comoglio PM, Giordano S, Trusolino L. Drug development of MET inhibitors: targeting oncogene addiction and expedience. Nature Reviews Drug Discovery; June 2008. 7(6):504-516.

3. Lutterbach B, et al. Lung Cancer Cell Lines Harboring MET Gene Amplification Are Dependent on Met for Growth and Survival. Cancer Research; March 1, 2007. 67(5):2081-2088.
4. Engelman JA, et al. MET Amplification Leads to Gefitinib Resistance in Lung Cancer by Activating ERBB3 Signaling. Science; May 18, 2007. 316:1039-1043.
5. Carson F, Hladik C. Histotechnology: A Self Instructional Text, 3rd edition. Hong Kong: American Society for Clinical Pathology Press; 2009.
6. Occupational Safety and Health Standards: Occupational exposure to hazardous chemicals in laboratories. (29 CFR Part 1910.1450). Fed. Register.
7. 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.
8. 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.

Symbols

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

GTIN	Global Trade Item Number
UDI	Unique Device Identification
	Indicates the entity importing the medical device into the European Union

REVISION HISTORY

Rev	Updates
B	Updates to Principle of the Procedure, Material Provided, Materials Required But Not Provided, Storage and Stability, Specimen Preparation, Warnings and Precautions, Staining Procedure, Negative Reagent Control, Positive Tissue Control, Performance Characteristics, References, Symbols, Intellectual Property, and Contact Information.

INTELLECTUAL PROPERTY

CONTACT INFORMATION

Ventana Medical Systems, Inc.
1910 E. Innovation Park Drive
Tucson, Arizona 85755
USA
+1 520 887 2155
+1 800 227 2155 (USA)

www.roche.com

EC REP

Roche Diagnostics GmbH
Sandhofer Strasse 116
D-68305 Mannheim
Germany
+800 5505 6606

CE