VENTANA TFF3 (7F1.21) Mouse Monoclonal Primary Antibody

REF 790-5059

07712812001

IVD Σ 50

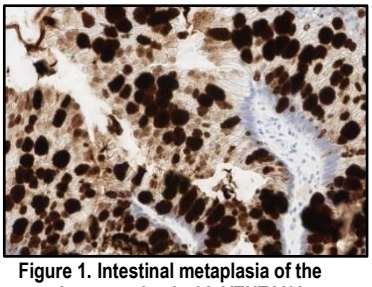

Figure 1. Intestinal metaplasia of the esophagus stained with VENTANA TFF3 (7F1.21) antibody.

INTENDED USE

The VENTANA TFF3 (7F1.21) Mouse Monoclonal Primary Antibody (VENTANA TFF3 (7F1.21)) is directed against a cytoplasmic epitope of the trefoil factor family 3 (TFF3) protein present in epithelial cells. This antibody may be used to aid in the identification of intestinal metaplasia (IM) of the esophagus. The antibody is intended for qualitative staining in sections of fixed, paraffin-embedded clinical samples stained with a BenchMark ULTRA automated staining instrument.

The clinical interpretation of any staining, or the absence of staining, must be complemented by histological/cytological 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

VENTANA TFF3 (7F1.21) antibody is a mouse monoclonal primary antibody produced against trefoil factor family 3 (TFF3) protein. Trefoil proteins are believed to have an important role in mucosal protection and repair in the esophagus.[1] TFF3 is a soluble peptide and a member of the trefoil peptide family, which is conserved among species and has a trefoil domain and C-terminal dimerization domain. TFF3 was first recognized in rat intestine, and was then shown to be primarily expressed and secreted onto the intestinal surface by goblet cells of the human small and large intestinal mucosa. TFF3 plays an important role in the reconstitution of the mucosal barrier to protect the epithelial layer against environmental injury.[2] TFF3 is predominantly expressed in the goblet cells of the small intestine and colon.[3]

PRINCIPLE OF THE PROCEDURE

VENTANA TFF3 (7F1.21) antibody is a mouse monoclonal primary antibody which binds to TFF3 in sections of formalin-fixed, paraffin-embedded (FFPE) samples. The specific antibody can be localized using a haptenated secondary antibody followed by a multimer anti-hapten-HRP conjugate (OptiView DAB IHC Detection Kit, Cat. No. 760-700 / 06396500001). The specific antibody-enzyme complex is then visualized with a precipitating enzyme reaction product. Refer to the OptiView DAB IHC Detection Kit insert for further information.

In addition to staining with VENTANA TFF3 (7F1.21) antibody, a second slide should be stained with Negative Control (Monoclonal) (Cat. No. 760-2014 / 05266670001).

MATERIAL PROVIDED

VENTANA TFF3 (7F1.21) antibody contains sufficient reagent for 50 tests.

One 5 mL dispenser of VENTANA TFF3 (7F1.21) antibody contains approximately 55 µg of a mouse monoclonal antibody.

The antibody is diluted in 0.08M PBS with 3% carrier protein and 0.05% ProClin 300, a preservative.

Specific antibody concentration is approximately 11 µg/mL. There is no known non-specific antibody reactivity observed in this product.

The TFF3 (7F1.21) antibody is a mouse monoclonal antibody produced as purified 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 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 package insert may be available in all geographies. Consult your local support representative.

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 samples are suitable for use with this primary antibody when used with VENTANA detection kits and BenchMark ULTRA instruments. The recommended fixative for histological samples is 10% neutral buffered formalin.[4] Cytological samples should be preserved per esophageal collection device manufacturer's instructions for use (IFU). Slides should be stained immediately, as antigenicity of cut sample 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. 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.
7. 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]
8. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
9. Avoid microbial contamination of reagents as it may cause incorrect results.
10. 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 dialog.roche.com.
11. Consult local and/or state authorities with regard to recommended method of disposal.
12. Product safety labeling primarily follows EU GHS guidance. Safety data sheet available for professional user on request.
13. 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.
	P261	Avoid breathing mist or vapours.
	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 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. Refer to Table 2 for the recommended staining protocol.

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 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 790-5059.

Table 2. Recommended Staining Protocol for VENTANA TFF3 (7F1.21) with U OptiView IHC DAB v5 on a BenchMark ULTRA instrument.

Procedure Type	Method
Deparaffinization	Selected
Cell Conditioning (Antigen Unmasking)	Cell Conditioning 1, 32 minutes
Antibody (Primary)	BenchMark ULTRA instrument 32 minutes, 36°C
OptiView HQ linker	8 minutes
OptiView HRP Multimer	8 minutes
Counterstain	Hematoxylin II, 4 minutes
Post Counterstain	Bluing, 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."[7]

POSITIVE TISSUE CONTROL

An example of a positive control tissue for this antibody is normal human small intestine tissue, which shows staining of the cytoplasm and/or membrane in goblet and glandular cells.

STAINING INTERPRETATION/ EXPECTED RESULTS.

The cellular staining pattern for VENTANA TFF3 (7F1.21) is cytoplasmic and/or membranous.

SPECIFIC LIMITATIONS

Histological samples should be fixed within 24 hours of collection with 10% neutral buffered formalin for 12-24 hours. Cytological samples should be preserved per esophageal collection device manufacturer's instructions for use (IFU).

This antibody has been optimized for a 32 minute incubation time on a BenchMark ULTRA instrument in combination with OptiView DAB IHC Detection Kit, but the user must validate results obtained with this reagent.

Sections approximately 4-6 µm in thickness should be cut and mounted on positively charged slides.

PERFORMANCE CHARACTERISTICS

Staining tests for specificity, sensitivity, and repeatability were conducted and the results are listed in Table 3 and Table 4 and in the Repeatability section. See Figures 1, 2 and 3 for staining pattern.

Specificity

Table 3. Specificity of VENTANA TFF3 (7F1.21) antibody was determined by testing FFPE normal tissues.

Tissue	# positive / total cases	Tissue	# positive / total cases
Cerebrum	0/3	Thymus gland	0/3
Cerebellum	0/3	Bone marrow	0/3
Adrenal gland	0/3	Lung	0/3
Ovary	0/3	Heart	0/3
Pancreas	0/3	Esophagus	1/19*
Lymph Node	0/3	Stomach	0/42
Hyphophysis	0/3	Small intestine	3/3**
Testis	0/3	Colon	3/3**
Thyroid	0/3***	Liver	0/3
Breast	0/3	Tongue	2/3*
Spleen	0/3	Kidney	0/3
Tonsil	0/3	Prostate	2/3*****
Endometrium	0/3	Uterus	0/1
Cervix	1/3****	Skin	0/3
Skeletal muscle	0/3	Peripheral nerve	0/3
Mesothelium (lung)	0/1	Mesothelium (heart)	0/2
Stomach-chronic gastritis with intestinal metaplasia	16/16**	Stomach-chronic gastritis	0/17
Esophagus/stomach with intestinal metaplasia	4/4**		

* Submucosal glandular cells and/or salivary glandular cells
** Goblet and glandular cells
*** Staining in follicular lumen (colloid)
**** Endocervical glands
***** Focal staining in prostatic glands

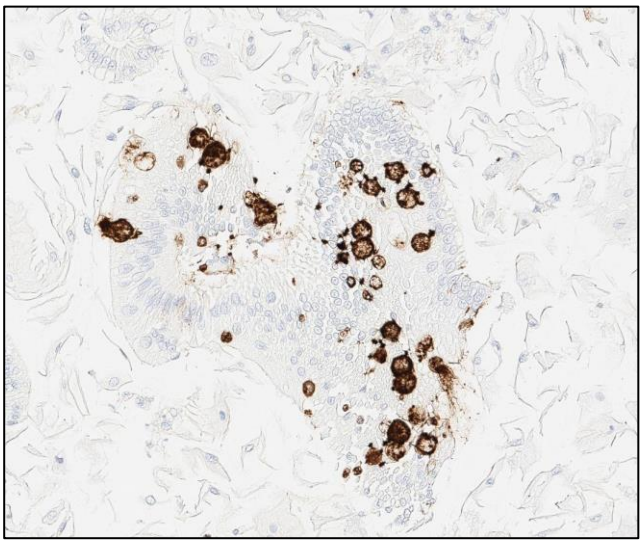

Figure 2. Presence of VENTANA TFF3 (7F1.21) antibody staining of intestinal metaplasia in cytology cell sample.

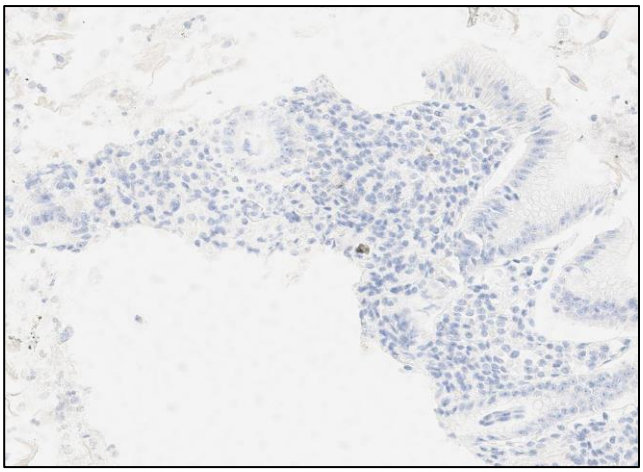

Figure 3. Absence of VENTANA TFF3 (7F1.21) antibody staining in cytology cell sample.

Sensitivity

Table 4. Sensitivity of VENTANA TFF3 (7F1.21) antibody was determined by testing a variety of FFPE neoplastic tissues.

Pathology	# positive / total cases
Glioblastoma	0/1
Atypical meningioma	0/1
Malignant ependymoma	0/1
Oligodendroglioma	0/1
Ovarian serous adenocarcinoma	1/1*
Ovarian adenocarcinoma	0/1
Islet cell tumor	0/1
Pancreatic adenocarcinoma	1/1
Seminoma	0/1
Medullary thyroid carcinoma	1/1
Papillary thyroid carcinoma	0/1
Breast-Invasive ductal carcinoma	1/1
Breast-Intraductal carcinoma	1/1
Breast-Invasive carcinoma	1/1
Splenic diffuse B-cell lymphoma	0/1
Pulmonary small cell undifferentiated carcinoma	0/1
Pulmonary squamous cell carcinoma	0/1
Pulmonary adenocarcinoma	0/1
Esophageal neuroendocrine carcinoma	0/1
Esophageal adenocarcinoma	0/1
Intestinal adenocarcinoma	1/1
Stromal sarcoma	0/1
Colorectal adenocarcinoma	2/2
Interstitialoma	0/2
Hepatocellular carcinoma	0/1
Hepatotoblastoma	0/1
Renal clear cell carcinoma	0/1
Prostatic adenocarcinoma	1/2
Leiomyoma	0/1
Endometrial adenocarcinoma	1/1
Uterine clear cell carcinoma	0/1
Cervical squamous cell carcinoma	0/2
Embryonal rhabdomyosarcoma	0/1
Malignant melanoma	N/A
Skin -Basal cell carcinoma	0/1
Skin -Squamous cell carcinoma	0/1
Neurofibroma	0/1
Neuroblastoma	0/1
Malignant mesothelioma	0/1
Diffuse B-cell lymphoma	0/2
Mixed type Hodgkin's lymphoma	0/1
Diffuse anaplastic large cell lymphoma	0/1
Bladder-Transitional cell carcinoma	0/1
Bladder-Low grade leiomyosarcoma	0/1

Pathology	# positive / total cases
Osteosarcoma	0/1
Spindle cell rhabdomyosarcoma	0/1
Intermediate grade leiomyosarcoma	0/1
Esophageal squamous cell carcinoma	0/9
Esophageal adenocarcinoma	8/15
Esophageal small cell carcinoma	1/6
Esophageal undifferentiated carcinoma	1/1
Stomach adenocarcinoma	80/218
Stomach adenocarcinoma, esophagogastric junction	36/85
Stomach adenocarcinoma, signet-ring cell carcinoma	9/16
Stomach adenocarcinoma, undifferentiated carcinoma	0/3
Stomach carcinoma , NOS	2/4

* Focal staining

Repeatability

Repeatability studies for VENTANA TFF3 (7F1.21) antibody were completed to demonstrate:

- Inter-lot reproducibility of the antibody.
- Intra-platform reproducibility on the BenchMark ULTRA instrument.
- Inter-platform reproducibility on the BenchMark ULTRA instrument.

All studies met their acceptance criteria.

REFERENCES

1. Dunn LJ, Jankowski JA, Griffin SM. Trefoil factor expression in a human model of the early stages of Barrett's Esophagus. Dig Dis Sci. 2014;60(5):1187-94.
2. Gu J, Zheng L, Zhang L, Chen S, Zhu M, Li X, Wang Y. TFF3 and HER2 expression and their correlation with survival in gastric cancer. Tumour Biol. 2015;36(4):3001-7.
3. Hoffmann W, Jagla W. Cell type specific expression of secretory TFF peptides: colocalization with mucins and synthesis in the brain. Int Rev Cytol. 2002;213:147-81.
4. Carson F, Hladik C. Histotechnology: A Self Instructional Text, 3rd edition. Hong Kong: American Society for Clinical Pathology Press; 2009.
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.

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
UDI	Unique Device Identification
	Indicates the entity importing the medical device into the European Union

REVISION HISTORY

Rev	Updates
B	Updates to Material Provided, Specimen Preparation, Warnings and Precautions, Symbols, and Contact Information

INTELLECTUAL PROPERTY

CONTACT INFORMATION

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