

VENTANA FGFR2b (FPR2-D) Mouse Monoclonal Antibody

REF

251-7236

10057939001



SUMMARY AND EXPLANATION

FGFR2b is a tyrosine kinase located in the membrane and functions as a receptor for proteins from the FGF family.^{1,2}

VENTANA FGFR2b (FPR2-D) Mouse Monoclonal Antibody is an immunohistochemical assay that utilizes a mouse monoclonal primary antibody (clone FPR2-D) for the detection of Fibroblast Growth Factor Receptor type 2b (FGFR2b) proteins, producing membranous staining in formalin-fixed, paraffin-embedded (FFPE) tissue. The specific antibody is visualized using OptiView DAB IHC Detection Kit (Cat. No. 760-700 / 06396500001) on a BenchMark IHC/ISH instrument.

Refer to the respective method sheet for further information.

For Research Use Only. Not for use in diagnostic procedures.

SPECIFICITY AND CLONE INFORMATION

Specificity: Human FGFR2b.

Clone #: FPR2-D

APPLICATION AND FORMULATION

Ready-to-Use format for use in IHC on FFPE tissue.

MATERIAL PROVIDED

VENTANA FGFR2b (FPR2-D) Mouse Monoclonal Antibody contains sufficient reagent for 50 tests.

One 5 mL dispenser of VENTANA FGFR2b (FPR2-D) Mouse Monoclonal Antibody contains approximately 15 µg of mouse monoclonal antibody.

This antibody contains 0.10% ProClin 300, a preservative.

Specific antibody concentration is approximately 3 µg/mL.

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 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:

- Human non-neoplastic skin tissue for use as positive tissue control
- Negative Control (Monoclonal) (Cat. No. 760-2014 / 05266670001)
- Microscope slides, positively charged
- OptiView DAB IHC Detection Kit (Cat. No. 760-700 / 06396500001)
- EZ Prep Concentrate (10X) (Cat. No. 950-102 / 05279771001)
- Reaction Buffer Concentrate (10X) (Cat. No. 950-300 / 05353955001)
- ULTRA LCS (Predilute) (Cat. No. 650-210 / 05424534001)
- LCS (Predilute) (Cat. No. 650-010 / 05264839001)
- ULTRA Cell Conditioning Solution (ULTRA CC1) (Cat. No. 950-224 / 05424569001)
- Cell Conditioning Solution (CC1) (Cat. No. 950-124 / 05279801001)
- Hematoxylin II (Cat. No. 790-2208 / 05277965001)
- Bluing Reagent (Cat. No. 760-2037 / 05266769001)
- Protease 3 (Cat. No. 760-2020 / 05266718001)
- General purpose laboratory equipment
- 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 a BenchMark IHC/ISH instrument. The recommended tissue fixative is 10% neutral buffered formalin, processed in accordance with standard practice.³ Fixatives such as alcohol-formalin-acetic acid (AFA), PREFER fixative and other alcohol-based fixatives may demonstrate a loss of specific staining for FGFR2b and should not be used with this assay. Alternative fixative types have not been assessed. Both sample and control tissues should be cut approximately 4 µm thick and mounted on positively charged glass microscope slides. Slides should be stained immediately, as antigenicity of cut tissue sections may diminish over time. Slides should be stored with desiccant and stored at room temperature.


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

WARNINGS AND PRECAUTIONS

- For Research Use Only (RUO).
- For professional use only.
- Do not use beyond the specified number of tests.
- 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.
- 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.
- 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.^{4,5}
- Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
- Avoid microbial contamination of reagents as it may cause incorrect results.
- For further information on the use of this device, refer to the instrument User Guide, and instructions for use of all necessary components located at navifyportal.roche.com.
- Consult local and/or state authorities with regard to recommended method of disposal.
- Product safety labeling primarily follows EU GHS guidance. Safety data sheet available for professional user on request.

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

Table 1. Hazard information.

Hazard	Code	Statement
	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, a reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1)

STAINING PROCEDURE

VENTANA assays are for use on BenchMark IHC/ISH instruments in combination with VENTANA detection kits and accessories. Refer to Table 2 for a recommended staining protocol.

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 251-7236.

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

Table 2. Recommended staining protocol for VENTANA FGFR2b (FPR2-D) Mouse Monoclonal Antibody and Negative Control (Monoclonal) with OptiView DAB IHC Detection Kit on a BenchMark IHC/ISH instrument.

Procedure Type	Method	
	GX	ULTRA or ULTRA PLUS [a]
Staining Procedure	RUO GX FGFR2b (FPR2-D)	RUO U FGFR2b (FPR2-D)
Cell Conditioning Enzyme ^[b]	Locked	Pre Cell Conditioning Enzyme or Post Cell Conditioning Enzyme
Cell Conditioning ^[c] (Antigen Unmasking)	CC1 24 Min or CC1 32 Min	Locked
Antibody (Primary)	FGFR2b (FPR2-D) Ab, Selected, 37C or Negative Control Ab, Selected, 37C	
Counterstain	Locked	Hematoxylin II, 4 minutes
Post Counterstain	Locked	Bleuing, 4 minutes

[a] Concordance was demonstrated between BenchMark ULTRA and BenchMark ULTRA PLUS instruments using representative assays.

[b] When Pre Cell Conditioning Enzyme is selected on the BenchMark ULTRA or ULTRA PLUS platform, the antibody incubation time will be automatically set at 32 minutes at 37C. When Post Cell Conditioning Enzyme is selected on the BenchMark ULTRA or ULTRA PLUS platform, the antibody incubation time will be automatically set at 48 minutes at 37C.

[c] When CC1 24 Min is selected on the BenchMark GX platform, the antibody incubation time will be automatically set at 32 minutes at 37C. When CC1 32 Min is selected on the BenchMark GX platform, the antibody incubation time will be automatically set at 48 minutes at 37C.

For further information on fixation variables, refer to "Immunohistochemistry Principles and Advances."⁶

NEGATIVE REAGENT CONTROL

A negative reagent control is a test specimen that is stained with a mouse monoclonal, negative control and serves as a test specimen control to evaluate nonspecific staining allowing accurate interpretation of specific FGFR2b staining on the respective VENTANA FGFR2b (FPR2-D) Mouse Monoclonal Antibody-stained test specimen slide. A matched negative reagent control should be run for every specimen to aid in the interpretation of results. The staining procedure for the negative reagent control should be equal to the primary antibody.

POSITIVE TISSUE CONTROL

A positive tissue control is a control tissue specimen that has been stained in the same manner as the research specimen and should be run alongside samples to monitor the proper functioning of the reagents and instrument within the staining run, which demonstrates the assay is performing suitably. Properly fixed human non-neoplastic skin tissue (with weak and moderate membrane staining) is recommended as the positive tissue control for VENTANA FGFR2b (FPR2-D) Mouse Monoclonal Antibody.

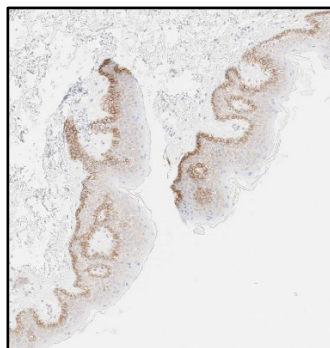


Figure 1. Non neoplastic skin (10x) as a positive control stains membranes with weak and moderate staining in the epithelium. Staining protocol RUO U FGFR2b (FPR2-D) Pre Cell Conditioning Enzyme, 32 minutes primary antibody incubation time.

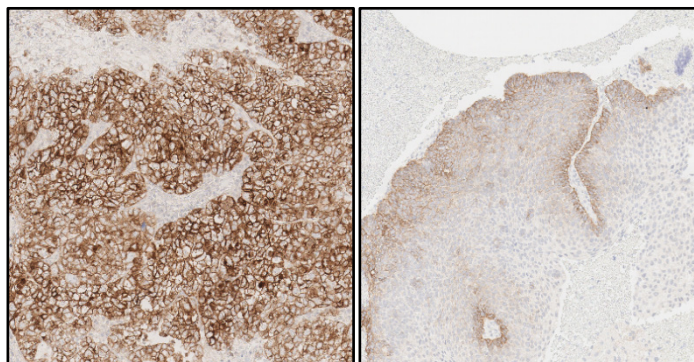


Figure 2. Non Small Cell Lung Carcinoma (NSCLC) metastasized to Uterus (10x Left) and Cervical Carcinoma (10x Right). Staining protocol RUO U FGFR2b (FPR2-D) Post Cell Conditioning Enzyme, 48 minutes primary antibody incubation time.

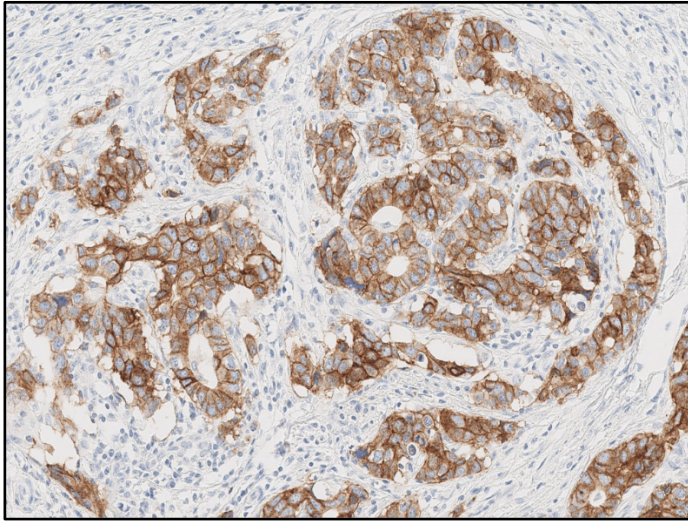


Figure 3. Gastric Carcinoma (20x) staining protocol RUO U FGFR2b (FPR2-D) Pre Cell Conditioning Enzyme, 32 minutes. primary antibody incubation time.

SPECIFIC LIMITATIONS

Immunohistochemistry is a multiple step process that requires specialized training in the selection of the appropriate reagents, tissue selections, fixation, processing, preparation of the immunohistochemistry slide, and interpretation of the staining results.

Tissue staining is dependent on the handling and processing of the tissue prior to staining. Improper fixation, freezing, thawing, washing, drying, heating, sectioning, or contamination with other tissues or fluids may produce artifacts, antibody trapping, or false negative results. Inconsistent results may result from variations in fixation and embedding methods, or from inherent irregularities within the tissue.

Fixatives such as alcohol-formalin-acetic acid (AFA), PREFER fixative and other alcohol-based fixatives may demonstrate a loss of specific staining for FGFR2b and should not be used with this assay.

TROUBLESHOOTING

1. If the positive control exhibits weaker staining than expected, other positive controls run during the same instrument run should be checked to determine if it is because of the primary antibody or one of the common secondary reagents.
2. If the positive control is negative, it should be checked to ensure that the slide has the proper bar code label. If the slide is labeled properly, other positive controls run on the same instrument run should be checked to determine if it is because of the primary antibody or one of the common secondary reagents. Tissues may have been improperly collected, fixed, or deparaffinized. The proper procedure should be followed for collection, storage and fixation.
3. If excessive background staining occurs, high levels of endogenous biotin may be present. Include a biotin blocking step in the staining protocol.
4. If all of the paraffin has not been removed, there may be no staining. Repeat the deparaffinization procedure.
5. If tissue sections wash off the slide, slides should be checked to ensure that they are positively charged.
6. For corrective action, refer to the instrument User Guide or contact your local support representative.
7. If a reagent dispenser does not dispense fluid, check the priming chamber or meniscus for foreign materials or particulates, such as fibers or precipitates. If the dispenser is blocked, do not use the dispenser and contact your local support representative. Otherwise, re-prime the dispenser by aiming the dispenser over a waste container, removing the nozzle cap, and pressing down on the top of the dispenser.

REFERENCES

1. Eswarakumar VP, Lax I, Schlessinger J. Cellular signaling by fibroblast growth factor receptors. *Cytokine Growth Factor Rev.* 2005;16(2):139-149
2. Jaye M, Schlessinger J, Dionne CA. Fibroblast growth factor receptor tyrosine kinases: molecular analysis and signal transduction. *Biochim Biophys Acta.* 1992;1135(2):185-199.
3. Carson FL, Cappellano C. *Histotechnology; A Self-Instructional Text*, 5th edition. American Society for Clinical Pathology Press; 2020, 2022.
4. Occupational Safety and Health Standards: Occupational exposure to hazardous chemicals in laboratories. (29 CFR Part 1910.1450). *Fed. Register.*
5. Directive 2000/54/EC of the European Parliament and Council of 24 June 2020 on the protection of workers from risks related to exposure to biological agents at work.
6. 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 elabdoc.roche.com/symbols for more information).



Global Trade Item Number



Unique Device Identification



Indicates the entity importing the medical device into the European Union

INTELLECTUAL PROPERTY

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