

## **Rabbit anti-human ROS1 (Proto-Oncogene Tyrosine-Protein Kinase ROS)** **Monoclonal Antibody (Clone D4D6)**

### REFERENCES AND PRESENTATIONS<sup>1</sup>

- **ready-to-use (ml)**  
MAD-000746QD-3  
MAD-000746QD-7  
MAD-000746QD-12
- **MD-Stainer presentations<sup>2</sup>**  
MAD-000746QD-3/V  
MAD-000746QD/V
- **concentrated**  
MAD-000746Q - 1:50 recommended dilution

### COMPOSITION

Anti-human ROS1 rabbit monoclonal antibody purified from serum and prepared in 10mM PBS, pH 7.4, with 0.2% BSA and 0.09% sodium azide

**INTENDED USE** : Immunohistochemistry (IHC) on paraffin embedded tissues. Not tested on frozen tissues or Western-Blotting

**CLONE:** D4D6

**Ig ISOTYPE:** Rabbit IgG

**IMMUNOGEN:** Synthetic protein corresponding to waste of the C-terminal domain of the human ROS1 protein.

**SPECIES REACTIVITY:** In vitro diagnostics in humans. Not tested in other species

### DESCRIPTION AND APPLICATIONS:

The transmembrane proto-oncogene tyrosine-kinase protein ROS, better known as ROS1, is a protein belonging to the insulin receptors tyrosine-kinase subfamily whose production is encoded by the ROS (MCF3) gene, located in the 6q22.1 chromosome region. After its activation, this gene intervenes in numerous molecular pathways related to the cell differentiation, proliferation, growth and survival including the activation of the PI3K-mTOR pathway. Chromosome aberrations affecting the ROS gene have been described in the glioblastoma multiforme through fusions of the C-terminal portion of ROS1 with the N-terminal domain of the FIG (Fused in Glioblastoma) encoded in the GOPC gene. The

chimeric protein GOPC-ROS1 is located at the level of the Golgi apparatus and presents activity as tyrosine kinase receptor. Other fusions of ROS1 with different genes such as SLC34A2, CD74, EZR, LRIG3, SDC4, TPM3, CCDC6 or KDEL2 among others, have as a result the appearance and expression of several chimeric proteins in 1-3 % of cases of lung adenocarcinoma and represents the therapeutic target for Crizotinib and analog molecules.

Non-specific staining on macrophages and reactive type II pneumocytes has also been described in isolated cases, whereas mucinous adenocarcinomas in general show low diffuse cytoplasmic staining in the absence of the translocation of the ROS1 gene.

In order to identify the cases of lung carcinomas with translocations of ROS1, complex techniques of RT-PCR and FISH can be used, although the rabbit monoclonal antibody D4D6 has been recently validated as a useful screening tool for positive cases of immunostaining against ROS1, which in comparative studies through in situ hybridization techniques with break-apart probes has proven a sensitivity and specificity of over 95%.

In order to consider a case as positive for ROS1, the immunostaining has to be strong or moderately intense on the membrane and/or the cytoplasm in more than 75% of tumor cells. Depending on the type of fusion of the ROS1 gene, other immunostaining patterns can be expressed as cytoplasmic punctiform in the CD74-ROS1 cases or cytoplasmic with linear accentuation in the lateral or apical membrane in the EZR-ROS1 cases. Although the morphology of the tumor cannot be considered as a criterion to select the positive ROS1 cases, the solid, micro-papillary, cribriform and signet ring cell growth patterns have been observed more frequently among the positive ROS1 cases. Focal and low-intensity staining can also be observed in up to 30% of the non-translocated cases. Therefore, the result must be confirmed with other analytical methods.

50% of the inflammatory myofibroblastic tumors also present translocation of the ALK gene, whereas the remaining ones show a little known genetic profile. It has been recently proven in a relatively low number of cases that up to 10% of these tumors show diffuse cytoplasmic or punctiform staining against the D4D6 clone (with translocation confirmed through FISH and RT-PCR techniques). In this same study, the antibody proved weak, focal nuclear staining in cases of gastrointestinal stromal tumors, myofibroblastic sarcomas, leiomyosarcomas and follicular dendritic cell sarcomas.

<sup>1</sup> These references are for presentation in vials of Low Density Polyethylene (LDPE) dropper. In case the products are used in automated stainers, a special reference is assigned as follows:

- / L: Cylindrical screw-cap vials (QD-3 / L, QD-7 / L, QD-12 / L).

- / N: Polygonal screw-cap vials (QD-3 / N, QD-7 / N, QD-12 / N).

For different presentations (references / volumes) please contact the supplier.

<sup>2</sup> For Technical specifications for MD-Stainer, please contact your distributor.



Less than 1% of colorectal adenocarcinomas and up to 5% of stomach adenocarcinomas can show translocations of the ROS1 gene and consequential cytoplasmic staining. The low percentage of colorectal adenocarcinomas makes the gene as a potential therapeutic target, but in the case of the stomach adenocarcinomas, it can represent a therapeutic alternative, since, in general, the positive ROS1 cases show a non-amplified phenotype of HER2 and MET.

**IHC POSITIVE CONTROL:** Normal stomach/cell cultures previously characterized.

**VISUALIZATION:** Membrane and cytoplasmic.

**IHC RECOMMENDED PROCEDURE:**

- 4µm thick section should be taken on charged slides; dry overnight at 60°C
- Deparaffinise, rehydrate and HIER (heat induced epitope retrieval) – boil tissue in the Pt Module using Master Diagnóstica Tris-Edta buffer pH9<sup>3</sup> for 20 min at 95°C. Upon completion rinse with 3-5 changes of distilled or deionised water followed by cooling at RT for 20 min
- Endogenous peroxidase block - Blocking for 10 minutes at room temperature using peroxidase solution (ref. MAD-021540Q-125)
- Primary antibody: incubate for 30 minutes [The antibody dilution (when concentrated) and protocol may vary depending on the specimen preparation and specific application. Optimal conditions should be determined by the individual laboratory]
- For detection use Master Polymer Plus Detection System (HRP) (DAB included; ref. MAD-000237QK)
- Counterstaining with haematoxylin and final mounting of the slide

**STORAGE AND STABILITY:** 🕒 up to 18 months;  
🧊 stored at 2-8°C. Do not freeze.

**WARNINGS AND PRECAUTIONS:**

1. Avoid contact of reagents with eyes and mucous membranes. If reagents come into contact with sensitive areas, wash with copious amounts of water.
2. This product is harmful if swallowed.
3. Consult local or state authorities with regard to recommended method of disposal.
4. Avoid microbial contamination of reagents.

**SAFETY RECOMMENDATIONS**

This product is intended for laboratory professional use only. The product is NOT intended to be used as a drug or for domestic purposes. The current version of the Safety Data Sheet for this product can be

downloaded by searching the reference number at [www.vitro.bio](http://www.vitro.bio) or can be requested at [regulatory.md@vitro.bio](mailto:regulatory.md@vitro.bio).

**BIBLIOGRAPHY**

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<sup>3</sup> Ref: MAD-004070R/D

