

NKX2.2 (EP336)

Rabbit anti-NKX2.2 Monoclonal Antibody (clone EP336)

REFERENCES AND PRESENTATIONS¹

- ready-to-use (manual or LabVision AutoStainer)
 MAD-000772QD–3
 MAD-000772QD–7
 MAD-000772QD–12
- Ready-to-use (MD-Stainer)² MAD-000772QD-3/V MAD-000772QD/V
- concentrated MAD-000772Q - 1:50 recommended dilution

COMPOSITION

Rabbit anti-NKX2.2 monoclonal antibody obtained from purified ascitic fluid 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: EP336

Ig ISOTYPE: IgG

IMMUNOGEN: synthetic peptide corresponding to the human NKX2.2 protein

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

DESCRIPTION AND APPLICATIONS:

The NKX2.2 antibody, also known as NK2 or NKX2B, is a nuclear transcription factor that belongs to the NK2 family of homeobox genes and is encoded in the chromosome region 20p11.22. This gene is involved in the development of beta cells of the pancreas that produce insulin, as well as in the neuroendocrine, glial, neuronal and oligodendroglial differentiation. Likewise, it is also involved in several process of diencephalic development and organization as well as in the control of the genes involved in the axonal orientation.

In normal mature tissues, the NKX2.2 antigen is expressed in the neuroendocrine cells of the islets of Langerhans of the pancreas, the enterochromaffin

cells of the gastrointestinal tract and the oligodendrocytes.

In neoplasias, the functional analysis has showed that the NKX2.2 gene is necessary for the oncogenic transformation of the Ewing's sarcoma. Thus, this antibody has proved its usefulness in the diagnosis of this tumor, where the presence of a minimum of 5% of tumor cells with moderate/intense nuclear staining is considered positive. In the Ewing's sarcoma, the NKX2.2 antibody shows a sensitivity of 93% and a specificity of 89%. Although the expression of the NKX2.2 antigen is closely related to the EWSR1-FLI1 fusion, the cases with EWSR1-ERG fusion have also given positive results.

Other sarcomas and small round cell tumors with positivity against the NKX2.2 antibody are the olfactory neuroblastoma and the teratocarcinosarcoma, as well as a small part of small cell carcinomas, the CIC-DUX4 sarcomas, the neuroblastoma, the synovial sarcoma, the mesenchymal chondrosarcoma or the melanomas.

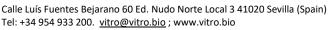
The NKX2.2 antibody also recognizes the gastrointestinal and pancreatic neuroendocrine tumors and, therefore, in conjunction with the PDX-1 and CDX2 markers, it has a diagnostic usefulness in the vast majority of neuroendocrine tumors.

IHC POSITIVE CONTROL: Tissue section from normal pancreas; Merkel cell carcinoma or Ewing sarcoma **VISUALIZATION**: Nuclear

IHC RECOMMENDED PROCEDURE:

- $4\mu m$ thick section should be taken on charged slides; dry overnight at $60^{\circ}C$
- Deparaffinise, rehydrate and HIER (heat induced epitope retrieval) boil tissue in the Pt Module using Vitro S.A EDTA buffer pH8³ 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]





¹ 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.

² For Technical specifications for MD-Stainer, please contact your distributor.

³ Ref: MAD-004072R/D



- 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: Stored at 2-8°C. Do not freeze. Once the packaging has been opened it can be stored until the expiration date of the reagent indicated on the label. If the reagent has been stored under other conditions to those indicated in this document, the user must first check its correct performance taking into account the product warranty is no longer valid.

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 <u>www.vitro.bio</u> or can be requested at regulatory@vitro.bio.

BIBLIOGRAPHY

1. Furuta H, Horikawa Y, Iwasaki N, Hara M, Sussel L, Le Beau MM, Davis EM, Ogata M, Iwamoto Y, German MS, Bell GI. Beta-cell transcription factors and diabetes: mutations in the coding region of the BETA2/NeuroD1 (NEUROD1) and Nkx2.2 (NKX2B) genes are not associated with maturity-onset diabetes of the young in Japanese. Diabetes. 1998 Aug;47(8):1356-8.

2. Smith R, Owen LA, Trem DJ, Wong JS, Whangbo JS, Golub TR, Lessnick SL. Expression profiling of EWS/FLI identifies NKX2.2 as a critical target gene in Ewing's sarcoma. Cancer Cell. 2006 May;9(5):405-16.

3. Yoshida A, Sekine S, Tsuta K, Fukayama M, Furuta K, Tsuda H. NKX2.2 is a useful immunohistochemical marker for Ewing sarcoma. Am J Surg Pathol. 2012 Jul;36(7):993-9.

4. Hung YP, Fletcher CD, Hornick JL. Evaluation of NKX2-2 expression in round cell sarcomas and other tumors with EWSR1 rearrangement: imperfect specificity for Ewing sarcoma. Mod Pathol. 2016 Apr;29(4):370-80.

5. Machado I, Yoshida A, López-Guerrero JA, Nieto MG, Navarro S, Picci P, Llombart-Bosch A.

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Immunohistochemical analysis of NKX2.2, ETV4, and BCOR in a large series of genetically confirmed Ewing sarcoma family of tumors. Pathol Res Pract. 2017 Sep;213(9):1048-1053.

6. Hung YP, Fletcher CD, Hornick JL. Evaluation of ETV4 and WT1 expression in CIC-rearranged sarcomas and histologic mimics. Mod Pathol. 2016 Nov;29(11):1324-1334.

7. Wang YC, Gallego-Arteche E, lezza G, Yuan X, Matli MR, Choo SP, Zuraek MB, Gogia R, Lynn FC, German MS, Bergsland EK, Donner DB, Warren RS, Nakakura EK. Homeodomain transcription factor NKX2.2 functions in immature cells to control enteroendocrine differentiation and is expressed in gastrointestinal neuroendocrine tumors. Endocr Relat Cancer. 2009 Mar;16(1):267-79.

8. McCuiston A, Bishop JA. Usefulness of NKX2.2 Immunohistochemistry for Distinguishing Ewing Sarcoma from Other Sinonasal Small Round Blue Cell Tumors. Head Neck Pathol. 2018 Mar;12(1):89-94.

LABEL AND BOX SYMBOLS

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IVD

Explanation of the symbols of the product label and box:

	Expiration date
Ĵ	Temperature limit
***	Manufacturer
Σ	Sufficient content for <n> assays</n>
REF	Catalog number
LOT	Lot code
Ĩ	Refer to the instructions of use
IVD	Medical product for <i>in</i> <i>vitro</i> diagnosis.
e-SDS	Material safety data sheet