

# Nerve Growth Factor Receptor (NGFR) (MRQ-21)

## Mouse Monoclonal Antibody



### Product Identification

Cat. No.	Description
44728	NGFR 0.1 M (MRQ-21)
44729	NGFR 1 M (MRQ-21)
44354	NGFR RTU M (MRQ-21)

### Symbol Definitions

<b>P</b>	ready-to-use
<b>C</b>	concentrate
<b>A</b>	ascites
<b>E</b>	serum
<b>S</b>	supernatant
<b>DIL</b>	concentrate dilution range
<b>DOC#</b>	document number
<b>DIS</b>	distributed by

### Intended Use

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

Nerve Growth Factor Receptor (NGFR) antibody is intended for qualified laboratories to qualitatively identify by light microscopy the presence of associated antigens in sections of formalin-fixed, paraffin-embedded tissue sections using IHC test methods. Use of this antibody is indicated, subsequent to clinical differential diagnoses of diseases, as an aid in the identification of Spindle cell and Central Nervous System tumors within the context of antibody panels, the patient's clinical history, and other diagnostic tests evaluated by a qualified pathologist.

### Summary And Explanation

NGFR, a 75 kd glycoprotein (also known as P-75NTR), is the first of neurotrophin receptors to be isolated and is a member of the tumor necrosis factor (TNF) receptor family. It is expressed not only in sympathetic and sensory neurons, but also in various neural crest cell or tumor derivatives such as melanocytes, melanomas, neuroblastomas, pheochromocytomas, neurofibromas, and

neurotized nevi (type C melanocytes). NGFR has been shown to be a reliable marker for desmoplastic and neurotropic melanoma by several groups. Although the sensitivity of NGFR staining in desmoplastic melanomas has not been systematically analyzed, all reported cases stain with NGFR. A review of 9 cases of desmoplastic melanomas diagnosed in the Dermatopathology Section of the Department of Dermatology at Boston University School of Medicine between 2001 and 2004 disclosed that all 9 cases of desmoplastic melanomas tested stained positively with NGFR. These findings are in contrast to a study reported by Huttenbach et al in which only 33% of their desmoplastic melanomas stained with NGFR. This difference emphasizes the importance of the choice of monoclonal antibody to obtain consistent results. This staining property of desmoplastic melanoma cells can be useful in diagnosing challenging cases, namely early lesions, suspected cases with negative or weak S-100 staining, and those that must be distinguished from a scar. It is now apparent that expression of NGFR is ubiquitous and not limited to the nervous system, being expressed in mature nonneural cells such as perivascular cells, dental pulp cells, lymphoidal follicular dendritic cells, basal epithelium of oral mucosa and hair follicles, prostate basal cells and myoepithelial cells. Unlike the high-affinity nerve growth factor tyrosine kinase receptors (TrkA, TrkB and TrkC), NGFR has no intrinsic tyrosine kinase activity. Studies in prostate and urothelial cancer suggest that NGFR may act as a tumour suppressor, negatively regulating cell growth and proliferation. Anti-NGFR labels the myoepithelial cells of breast ducts and intralobular fibroblasts of breast ducts and thus aides in the diagnosis of malignancy in the breast. Associated products: GFAP, S.M. Myosin, Calponin, NSE, Myelin Basic Protein, S-100, HMB45, Tyrosinase, MART-1

### Principles And Procedures

The stated primary antibody may be used as the primary antibody for immunohistochemical staining of formalin-fixed, paraffin-embedded tissue sections. In general, immunohistochemical staining in conjunction with a streptavidin-biotin detection system

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allows the visualization of antigens via the sequential application of a specific antibody (primary antibody) to the antigen, a secondary antibody (link antibody) to the primary antibody, an enzyme complex and a chromogenic substrate with interposed washing steps. Alternatively, a biotin-free polymer detection system may be used. The enzymatic activation of the chromogen results in a visible reaction product at the antigen site. The specimen may then be counterstained and a coverslip applied. Results are interpreted using a light microscope and aid in the differential diagnosis of pathophysiological processes, which may or may not be associated with a particular antigen.

Prediluted products are optimally diluted for use with a wide variety of detection kits offered by other manufacturers.

### Materials And Methods

**See product label for lot specific information for the following:**

1. Antibody immunoglobulin concentration
2. Source details

#### Reagents Provided

**Prediluted** The stated primary antibody product contains ready-to-use reagent.

The predilute immunoglobulin concentration range for this product is 0.5-2.5 µg/ml.

**Concentrated** The stated primary antibody product contains concentrated reagent.

Both the prediluted and concentrated formats of this antibody are diluted in Tris Buffer, pH 7.3-7.7, with 1% BSA and <0.1% Sodium Azide.

The concentrate immunoglobulin concentration range for this product is 80-160 µg/ml.

The recommended working dilution range for the concentrate product is 1:100-1:500 and can be found on the product label.

Isotype: IgG<sub>1</sub>

#### Reconstitution, Mixing, Dilution, Titration

Prediluted antibody is ready-to-use and optimized for staining. No reconstitution, mixing, dilution, or titration is required. The concentrated antibody is optimized to be diluted to within the dilution range.

The user must validate the working dilution of the concentrated product. Differences in tissue processing and technical procedures in the laboratory may produce significant variability in results and consequently require regular use of controls. (See Quality Control Procedures section)

#### Materials And Reagents Needed But Not Provided

The following reagents and materials may be required for staining but are not provided with the primary antibody:

1. Positive and negative control tissue
2. Microscope slides, positively charged
3. Drying oven capable of maintaining a temperature of 58-60°C ± 5°C
4. Staining jars or baths
5. Timer
6. Xylene or xylene substitute
7. Ethanol or reagent alcohol
8. Deionized or distilled water
9. Electric Pressure Cooker for tissue pretreatment step
10. Detection system and chromogen
11. Wash Solutions
12. Hematoxylin or other counterstain
13. Antibody diluents
14. Peroxide Block for use with HRP
15. Avidin-Biotin Block
16. Negative Control Reagent
17. Mounting medium
18. Cover glass
19. Light microscope (40-400x)

#### Storage and Handling

Store at 2-8°C. Do not freeze.

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

Every antibody reagent 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 for the prescribed storage method.

There are no definitive signs to indicate instability of this product; therefore, positive and negative controls should be run simultaneously with unknown specimens. Contact A.Menarini Diagnostics customer service if there is a suspected indication of reagent instability.

#### Specimen Collection and Preparation for Analysis

Routinely processed, neutral-buffered formalin-fixed, paraffin-embedded, tissues are suitable for use with this primary antibody. The recommended tissue fixative is 10% neutral-buffered formalin. Variable results may occur as a result of prolonged fixation or special processes such as decalcification of bone marrow preparations.

Each section should be cut to the appropriate thickness (approximately 3 µm) and placed on a positively charged glass slide. Slides containing the tissue section may be baked for at least 2 hours (but not longer than 24 hours) in a 58-60°C ± 5°C oven.

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### Warnings And Precautions

1. Take reasonable precautions when handling reagents. Use disposable gloves and lab coats when handling suspected carcinogens or toxic materials (example: xylene).
2. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
3. Patient specimens and all materials contacting them should be handled as biohazardous materials and disposed of with proper precautions. Never pipette by mouth.
4. Avoid microbial contamination of reagents, as this could produce incorrect results.
5. The user must validate incubation times and temperatures.
6. The prediluted, ready-to-use reagents are optimally diluted, and further dilution may result in loss of antigen staining.
7. The concentrated reagents may be diluted optimally based on validation by user. Any diluent used that is not specifically recommended herein must likewise be validated by the user for both its compatibility and effect on stability.
8. When used according to instructions, this product is not classified as a hazardous substance. The preservative in the reagent is less than 0.1% sodium azide and does not meet the EU Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) criteria for hazardous substance at the stated concentration.
9. The user must validate any storage conditions other than those specified in the package insert.
10. Diluent may contain bovine serum albumin and supernatant may contain bovine serum. The products containing fetal bovine serum and products containing bovine serum albumin are purchased from commercial suppliers. Certificates of Origin for the animal source used in these products are on file at Cell Marque. The certificates support that the bovine sources are from countries with negligible BSE risk and state sources of bovine from USA and Canada.
11. As with any product derived from biological sources, proper handling procedures should be used.

### Instructions For Use

#### Step By Step Procedure

Recommended Staining Protocols for the stated primary antibody:

#### Manual Staining

1. Deparaffinize, rehydrate, and epitope retrieve; the preferred method is the use of Heat Induced Epitope Retrieval (HIER) techniques. The preferred method allows for simultaneous deparaffinization, rehydration, and epitope retrieval. Upon completion, rinse with 5 changes of distilled or deionized water.
2. If using HRP detection system, place slides in a peroxide block for 10 minutes; rinse. If using AP detection system, omit this step.
3. Apply the antibody and incubate for 10 - 30 minutes; rinse.
4. For a 2 step polymer detection, system apply amplifier according to the manufacturer's instructions.
5. Apply the detection reagent for the manufacturer's recommended number of minutes, rinse.

6. Apply ample amount of chromogen and incubate for 1 - 10 minutes; rinse.
7. Dehydrate and coverslip

### Quality Control Procedures

#### Positive Tissue Control

A positive tissue control must be run with every staining procedure performed. This tissue may contain both positive and negative staining cells or tissue components and serve as both the positive and negative control tissue. Control tissues should be fresh autopsy, biopsy or surgical specimens prepared or fixed as soon as possible in a manner identical to the test sections. Use of a tissue section fixed or processed differently from the test specimen will serve to provide control for all reagents and method steps except fixation and tissue processing.

A tissue with weak positive staining is more suitable for optimal quality control and for detecting minor levels of reagent degradation. Positive tissue control for the stated primary antibody may include the following:

Positive Tissue Control	
Tissue	Visualization
Breast	Cytoplasmic

Known positive tissue controls should be utilized only for monitoring the correct performance of processed tissues and test reagents, not as an aid in determining a specific diagnosis of patient samples. If the positive tissue controls fail to demonstrate appropriate positive staining, results with the test specimens must be considered invalid.

#### Negative Tissue Control

The same tissue used for the positive tissue control may be used as the negative tissue control. The variety of cell types present in most tissue sections offers internal negative control sites, but this should be verified by the user. The components that do not stain should demonstrate the absence of specific staining, and provide an indication of non-specific background staining. If specific staining occurs in the negative tissue control sites, results with the patient specimens must be considered invalid.

#### Unexplained Discrepancies

Unexplained discrepancies in controls should be referred to A.Menarini Diagnostics Customer Service immediately. If quality control results do not meet specifications, patient results are invalid. See the Troubleshooting section of this insert. Identify and correct the problem, then repeat the entire procedure with the patient samples.

#### Negative Control Reagent

A negative control reagent must be run for every specimen to aid in the interpretation of results. A negative control reagent is used in place of the primary antibody to evaluate nonspecific staining. The slide should be treated with negative control reagent, matching the host species of the primary antibody, and ideally having the same IgG concentration. The incubation period for the negative control reagent should equal the primary antibody incubation period.

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### Interpretation Of Results

The immunostaining procedure causes a colored reaction product to precipitate at the antigen sites localized by the primary antibody. Refer to the appropriate detection system package insert for expected color reactions. A qualified pathologist experienced in immunohistochemistry procedures must evaluate positive and negative tissue controls before interpreting results.

#### Positive Tissue Control

The stained positive tissue control should be examined first to ascertain that all reagents are functioning properly. The presence of an appropriately colored reaction product within the target cells is indicative of positive reactivity. Refer to the package insert of the detection system used for expected color reactions. Depending on the incubation length and potency of the hematoxylin used, counterstaining will result in a pale to dark blue coloration of cell nuclei. Excessive or incomplete counterstaining may compromise proper interpretation of results. If the positive tissue control fails to demonstrate appropriate positive staining, any results with the test specimens are considered invalid.

#### Negative Tissue Control

The negative tissue control should be examined after the positive tissue control to verify the specific labeling of the target antigen by the primary antibody. The absence of specific staining in the negative tissue control confirms the lack of antibody cross reactivity to cells or cellular components. If specific staining occurs in the negative tissue control, results with the patient specimen are considered invalid. Nonspecific staining, if present, will have a diffuse appearance. Sporadic light staining of connective tissue may also be observed in sections from tissues that are not optimally fixed. Intact cells should be used for interpretation of staining results. Necrotic or degenerated cells show non-specific staining.

#### Patient Tissue

Patient specimens should be examined last. Positive staining intensity should be assessed within the context of any background staining of the negative reagent control. As with any immunohistochemical test, a negative result means that the antigen in question was not detected, not that the antigen is absent in the cells or tissue assayed. A panel of antibodies may aid in the identification of false negative reactions (see Summary of Expected Results section). The morphology of each tissue sample should also be examined utilizing a hematoxylin and eosin stained section when interpreting any immunohistochemical result. The patient's morphologic findings and pertinent clinical data must be interpreted by a qualified pathologist.

### Limitations

1. This reagent is "for professional use only" as immunohistochemistry is a multiple step process that requires specialized training in the selection of the appropriate reagents, tissues, fixation, processing; preparation of the immunohistochemistry slide; and interpretation of the staining results.
2. For laboratory use only.
3. For *in vitro* diagnostic use.
4. 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, as well as from inherent irregularities within the tissue.
5. Excessive or incomplete counterstaining may compromise proper interpretation of results.
6. The clinical interpretation of any positive staining, or its absence, must be evaluated within the context of clinical history, morphology, other histopathological criteria as well as other diagnostic tests. This antibody is intended to be used in a panel of antibodies if applicable. It is the responsibility of a qualified pathologist to be familiar with the antibodies, reagents, diagnostic panels, and methods used to produce the stained preparation. Staining must be performed in a certified, licensed laboratory under the supervision of a pathologist who is responsible for reviewing the stained slides and assuring the adequacy of positive and negative controls.
7. Ready to use antibodies and reagents are provided at optimal dilution for use as instructed. Any deviation from recommended test procedures may invalidate expected results. Appropriate controls must be employed and documented. Users in any circumstance must accept responsibility for interpretation of patient results.
8. Concentrated products are provided so that the user may subsequently optimally dilute for use subject to the user's determination of and adherence to suitable validation techniques. Users must validate the use of any diluents other than what is recommended herein. Once the primary is validated to be suitable for use, any deviation from recommended test procedures may invalidate expected results. Appropriate controls must be employed and documented. Users in any circumstance must accept responsibility for interpretation of patient results.
9. This product is not intended for use in flow cytometry.
10. Reagents may demonstrate unexpected reactions in previously untested tissues. The possibility of unexpected reactions even in tested tissue groups cannot be completely eliminated because of biological variability of antigen expression in neoplasms, or other pathological tissues. Contact A.Menarini Diagnostics customer service with any suspected, documented unexpected reactions.
11. Tissues from persons infected with hepatitis B virus and containing hepatitis B surface antigen (HBsAg) may exhibit nonspecific staining with horseradish peroxidase.
12. When used in blocking steps, normal sera from the same animal source as the secondary antisera may cause false negative or false positive results because of the effect of autoantibodies or natural antibodies.
13. False positive results may be seen because of nonimmunological binding of proteins or substrate reaction products. They may also be caused by pseudoperoxidase activity (erythrocytes), endogenous peroxidase activity (cytochrome C), or endogenous biotin (example: liver, brain, breast, kidney) subject to the type of immunostaining technique used.
14. As with any immunohistochemistry test, a negative result means that the antigen was not detected, not that the antigen was absent in the cells or tissue assayed.

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### Specific Limitations

1. The prediluted antibody products are optimized as a ready-to-use product. Because of the possibility of variation in tissue fixation and processing, it may be necessary to increase or decrease the primary antibody incubation time on individual specimens.
2. The antibody, in combination with detection systems and accessories, detects antigen(s) that survive routine formalin fixation, tissue processing and sectioning. Users who deviate from recommended test procedures remain, as they would in any circumstance, responsible for interpretation and validation of patient results.

### Summary of Expected Results

See the following tables of reactivity:

Normal Study			
Tissue	Positive Cases	Total Cases Tested	Notes
Brain	1	1	
Adrenal Cortex	1	1	
Ovary	0	1	
Pancreas	1	2	
Parathyroid	0	1	
Pituitary	1	1	
Testis	0	1	
Thyroid	1	1	
Breast	2	2	
Spleen	0	1	
Tonsil	0	1	
Thymus	0	1	
Bone Marrow	0	2	
Lung	0	2	
Heart	1	1	
Esophagus	0	1	
Stomach	0	1	
Small Intestine	0	1	
Colon	0	1	
Liver	0	1	
Salivary Gland	0	2	
Gall Bladder	1	1	
Kidney	1	1	
Bladder	0	1	
Prostate	1	2	
Uterus	2	2	
Fallopian Tube	1	1	
Ureter	1	1	
Cervix	1	1	
Skeletal Muscle	0	2	
Smooth Muscle	0	4	
Skin	2	2	
Peripheral Nerve	1	1	
Mesothelium	0	1	

Normal Study (continued)			
Tissue	Positive Cases	Total Cases Tested	Notes
Fat	0	1	
Placenta	1	1	

Disease Tissue Study			
Tissue	Positive Cases	Total Cases Tested	Notes
Breast invasive ductal carcinoma	0	8	
Colon adenocarcinoma	1	18	
Melanoma	1	1	

### Troubleshooting

1. If the positive control exhibits weaker staining than expected, other positive controls run during the same staining 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, other positive controls used on the same run should be checked to determine if the underlying cause relates to 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. A biotin blocking step should be included unless a biotin-free detection system is being used in which case any biotin present would not be a contributing factor to background staining.
4. If all of the paraffin has not been removed, the deparaffinization procedure should be repeated.
5. If tissue sections wash off the slide, slides should be checked to ensure that they are positively charged. Other possibilities that could have adverse affect on tissue adhesion include insufficient drying of the tissue section on the slide prior to staining or fixation in formalin that was not properly neutral-buffered. Tissue thickness may also be a contributing factor.

For corrective action, refer to the Step by Step Procedure section or contact A.Menarini Diagnostics customer service.

### Reference

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- 3 Liang Y, Johansson O. Light and electron microscopic demonstration of the p75 nerve growth factor receptor in normal human cutaneous nerve fibers: new vistas. *J Invest Dermatol.* 1998 Jul;111(1):114-8.
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