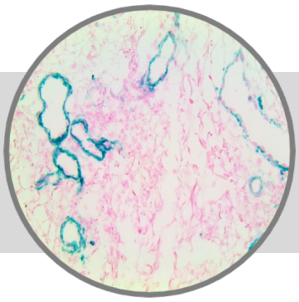
PI 3696, Rev. A DCN: 4104

TintoFast NGFR

Clone: BSB-18

Mouse Monoclonal



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Inset: IHC of NGFR on a Frozen Acetone-fixed Squamous Cell Carcinoma Tissue

Intended Use

For Mohs In Vitro Diagnostic Use.

This antibody is intended for the fast immunohistochemical detection of melanocytes or melanoma cells during intraoperative Mohs surgery on frozen sections. Additionally, this antibody can also be used on FFPE specimens. Interpretation of results should be performed by a qualified medical professional.

Immunogen

Recombinant human p75 nerve growth factor receptor protein.

Summary and Explanation

NGFR (Nerve Growth Factor Receptor), also termed p75 or CD271, is the low-affinity NGFR (LNGFR) which binds NGF and other neurotrophins, including BDNF, NT3 and NT4/5 with similar low-affinity. NGFR p75 is a 75 kD transmembrane glycoprotein that is mainly expressed in Schwann cells and neurons and in a variety of nonneuronal cells. NGFR p75 is necessary for regulating neuronal growth, migration, differentiation and cell death during development of the central and peripheral nervous system. NGFR p75 plays a central role in the regulation of cell number by apoptosis in the developing CNS. During early development, activation of NGFR p75 by NGF induces apoptotic cell death in some neuronal cells, probably through activation of the sphingomyelinase/ceramide pathway, the ICE-like proteases and the JNK pathway. CD271 has recently been described as being expressed in mesenchymal stem cells (bone marrow stromal cells).

NGFR 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). It is now apparent that expression of NGFR is ubiquitous and not limited to the nervous system. Studies in Prostate and Urothelial Cancer suggest that NGFR may act as a tumor suppressor, negatively regulating cell growth and proliferation. NGFR labels the myoepithelial cells of breast ducts and intralobular fibroblasts of breast ducts and, thus, aids in the diagnosis of malignancy in the breast.

Antibody Type	Mouse Monoclonal	**Clone**	BSB-18
Isotype	IgG1	**Reactivity**	Paraffin, Frozen
Localization	Cytoplasmic	**Control**	Brain, Breast, Prostate, Neuroblastoma, CNS Tumor
Species Reactivity		Human	

Product Limitations

Due to inherent variability present in immunohistochemical procedures (including fixation time of tissues, dilution factor of antibody, retrieval method utilized and incubation time), optimal performance should be established through the use of positive and negative controls. Results should be interpreted by a qualified medical professional.

Presentation

TintoFast NGFR is a mouse monoclonal antibody derived from cell culture supernatant that is concentrated, dialyzed, filter sterilized and diluted in buffer pH 7.5, containing BSA and sodium azide as a preservative.

Catalog No.	*Antibody Type*	*Dilution*	*Volume/Qty*
BSB-3696-3	TintoFast Predilute	Ready-to-Use	3.0 mL
BSB-3696-7	TintoFast Predilute	Ready-to-Use	7.0 mL
BSB-3696-15	TintoFast Predilute	Ready-to-Use	15.0 mL

Storage

Store at 2-8^{o}C

Stability

This product is stable up to the expiration date on the product label. Do not use after expiration date listed on package label. Temperature fluctuations should be avoided. Store appropriately when not in use and avoid prolonged exposure to room temperature conditions.

Precautions

1. For professional users only. Results should be interpreted by a qualified medical professional.
2. This product contains <0.1% sodium azide (NaN_3) as a preservative. Ensure proper handling procedures are used with this reagent.
3. Always wear personal protective equipment such as laboratory coat, goggles and gloves when handling reagents.
4. Dispose of unused solution with copious amount of water.
5. Do not ingest reagent. If reagent is ingested, seek medical advice immediately.
6. Avoid contact with eyes. If contact occurs, flush with large quantities of water.
7. Follow safety precautions of the heating device used for epitope retrieval (TintoRetriever Pressure Cooker or similar).
8. For additional safety information refer to Safety Data Sheet for this product.
9. For complete recommendations for handling biological specimens, please refer to the CDC document, "Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories" (see References in this document).

Specimen Preparation

Mohs Frozen sections and cell preparations: The antibody can be used for the detection of melanocytes and melanoma cells in Mohs acetone-fixed frozen sections.

Paraffin sections: The antibody can be used on formalin-fixed paraffin-embedded (FFPE) tissue sections. Ensure tissue undergoes appropriate fixation for best results. Pre-treatment of tissues with heat-induced epitope retrieval (HIER) is recommended using Bio SB ImmunoDNA Retriever with Citrate (BSB 0020-BSB 0023), ImmunoDNA Retriever with EDTA (BSB 0030-BSB 0033). Tissue should remain hydrated via use of Bio SB Immuno/DNA Washer solutions (BSB 0029 & BSB 0042).

Mohs IHC Procedure

Specimen Preparation of Mohs Frozen Tissues

1. Embed the specimen in OCT inside a cryostat.
2. Cut sections at 4-5 µm and mount on a positively charged glass slide such as the Bio SB Hydrophilic Plus Slides (BSB 7028).
3. Air dry the slide at room temperature for 2 minutes and then incubate the slide at 60 °C for 3 minutes in an incubator or dry bath.
4. Fix in 100% acetone for 2 minutes at room temperature.
5. Rinse with distilled water and air dry the slides for another 2 minutes at room temperature.

Tissue Pretreatment Procedure for Mohs Frozen Tissues

1. Subject tissues to epitope retrieval using Bio SB ImmunoDNA Retriever with Citrate (BSB 0020-BSB 0023), ImmunoDNA Retriever with EDTA (BSB 0030-BSB 0033). It is recommended that user uses a 5 minutes heat-induced epitope retrieval (HIER) method using a pressure cooker (BSB TintoRetriever Pressure Cooker) at 110°C.
2. Any of three HIER methods may be used:

a. TintoRetriever Pressure Cooker or Equivalent

Place tissues/slides in a staining dish or coplin jar containing the ImmunoDNA Retriever with Citrate or EDTA, and place on trivet or staining dish support in the pressure cooker. Add 1-2 inches of distilled water to the pressure cooker and turn heat to high (110-121° C). Incubate for 5 minutes. Open and immediately transfer slides to room temperature. Cool off for 5 -10 min

b. TintoRetriever PT Module or Water Bath Method

Place tissues/slides in a pre-warmed staining dish or coplin jar containing the ImmunoDNA Retriever with Citrate or EDTA at 95°-99° C. Incubate for 10-15 minutes.

c. Conventional Steamer Method

Place tissues/slides in a pre-warmed staining dish or coplin jar containing the ImmunoDNA Retriever with Citrate or EDTA in a steamer, cover and steam for 10-15 minutes.

IHC Detection Procedure

1. After HIER, transfer slides to ImmunoDNA washer and let stand for 1-2 minutes.
2. For manual staining, perform antibody incubation at ambient temperature. For automated staining methods, perform antibody incubation according to instrument manufacturer's instructions.
3. Wash slides with ImmunoDNA washer or DI water.
4. Continue IHC detection protocol. Wash slides between each step with ImmunoDNA washer solution.

Abbreviated Mohs Immunohistochemical Protocol

For best results we recommend using Mohs Frozen sections fixed with acetone for 2 min. then HIER with Citrate for 5 min at 110-121 ° C.

Step	Mohs PolyDetector HRP Green or DAB 5 min Protocol	Mohs PolyDetector HRP Green or DAB 10 min Protocol
Peroxidase/AP Blocker	0.5 min.	0.5 min.
Primary Antibody	2 min	4 min.
1st Step Detection	1 min	3 min.
Substrate-Chromogen	1 min	2 min.
Counterstain / Coverslip	0.5 min	0.5 min.

This protocol can also be used with FFPE Tissues retrieved with Citrate or EDTA.

Specimen Preparation for FFPE Tissues

1. Cut and mount 3-5-micron formalin-fixed paraffin-embedded tissues on positively charged slides such as Bio SB Hydrophilic Plus Slides (BSB 7028).
2. Air dry for 2 hours at 58° C.

Tissue Pretreatment Procedure for FFPE Tissues

1. Deparaffinize and rehydrate tissues.
2. Subject tissues to heat induced epitope retrieval (HIER) using a suitable retrieval solution such as ImmunoDNA Retriever with Citrate (BSB 0020-BSB 0023) or EDTA (BSB 0030-BSB 0033).
3. Any of three heating methods may be used:

a. TintoRetriever Pressure Cooker or Equivalent

Place tissues/slides in a staining dish or coplin jar containing the ImmunoDNA Retriever with Citrate or EDTA, and place on trivet in the pressure cooker. Add 1-2 inches of distilled water to the pressure cooker and turn heat to high. Incubate for 10-15 minutes. Open and immediately transfer slides to room temperature.

b. TintoRetriever PT Module or Water Bath Method

Place tissues/slides in a pre-warmed staining dish or coplin jar containing the ImmunoDNA Retriever with Citrate or EDTA at 95°-99° C. Incubate for 20-30 minutes.

c. Conventional Steamer Method

Place tissues/slides in a pre-warmed staining dish or coplin jar containing the ImmunoDNA Retriever with Citrate or EDTA in a steamer, cover and steam for 20-30 minutes.

4. After heat treatment, transfer slides in ImmunoDNA Retriever with Citrate or EDTA to room temperature and let stand for 10 minutes.
5. For manual staining, perform antibody incubation at ambient temperature. For automated staining methods, perform antibody incubation according to instrument manufacturer's instructions.
6. Wash slides with ImmunoDNA washer or DI water.
7. Continue Mohs IHC staining protocol. Wash slides between each step with ImmunoDNA washer solution.

Mounting Protocols

Mount with aqueous mounting such as AquaMounter (BSB-0090- BSB 0093) or permanent mounting media such as XyGreen PermaMounter (BSB 0169-0174) or organic solvent-based resin such as PermaMounter (BSB 0094-0097).

References

1. Liang Y, Johansson O, J Invest Dermatol. 1998;Jul;111(1):114-8
2. Radfar A, et al. Am J Dermatopathol. 2006;Apr;28(2):162-7
3. U.S. Department of Health and Human Services: Centers for Disease Control and Prevention. Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories. Supplement / Vol. 61, January 6, 2012.

Symbol Key / Légende des symboles/Erläuterung der Symbole

	Storage Temperature Limites de température Zulässiger Temperaturbereich	Manufacturer Fabricant Hersteller	REF Catalog Number Référence du catalogue Bestellnummer
IVD In Vitro Diagnostic Medical Device Dispositif médical de diagnostic in vitro In-Vitro-Diagnostikum	Read Instructions for Use Consulter les instructions d'utilisation Gebrauchsanweisung beachten	Expiration Date Utiliser jusque Verwendbar bis	LOT Lot Number Code du lot Chargenbezeichnung

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