

Inset: IHC of Alpha Synuclein on a FFPE Astrocytoma Tissue

Intended Use

For In Vitro Diagnostic Use.

This antibody is intended for use in Immunohistochemical applications on formalinfixed paraffin-embedded tissues (FFPE), frozen tissue sections and cell preparations. Interpretation of results should be performed by a qualified medical professional.

Immunogen

Recombinant protein of the human alpha synuclein.

Summary and Explanation

Alpha-synuclein is a 140 amino acids protein encoded by the SNCA gene. It is predominantly expressed in the neocortex, hippocampus, substantia nigra, thalamus, and cerebellum with smaller amounts are found in the heart, muscles, and other tissues. In the brain, alpha-synucle-in is found mainly in presynaptic terminals.

An alpha-synuclein fragment, known as the non-Abeta component (NAC) of Alzheimer's disease amyloid, originally found in an amyloid-enriched fraction, was shown to be a fragment of its precursor protein, NACP. Alpha-synuclein aggregates to form insoluble fibrils in pathological conditions characterized by Lewy bodies, such as Parkinson's disease, dementia with Lewy bodies and multiple system atrophy. These disorders are known as synucleinopathies. Occasionally, Lewy bodies contain tau protein; however, alpha-synuclein and tau constitute two distinctive subsets of filaments in the same inclusion bodies.

Alpha-synuclein pathology is also found in both sporadic and familial cases with Alzheimer's disease. In rare cases of familial forms of Parkinson's disease, there is a mutation in the gene coding for alpha-synuclein. Genomic duplication and triplication of the gene appear to be a rare cause of Parkinson's disease in other lineages, although more common than point mutations. Hence certain mutations of alpha-synuclein may cause it to form amyloid-like fibrils that contribute to Parkinson's disease.

Antibody Type	Mouse Monoclonal	Clone BSB-114			
lsotype	lgG2a/K	Reactivity	Paraffin, Frozen		
Localization	Cytoplasmic, Nuclear	Control	Brain, Breast, Skin, Tonsil, Bone Mar- row, Alzheimer's Disease, Parkinson's Disease		
Species Reactivity Human					

Presentation

Alpha Synuclein 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 Num.	Antibody Type	Dilution	Volume/Qty
BSB 3286	Tinto Prediluted	Ready-to-Use	3.0 mL
BSB 3287	Tinto Prediluted	Ready-to-Use	7.0 mL
BSB 3288	Tinto Prediluted	Ready-to-Use	15.0 mL
BSB 3289	Concentrated	1:25 - 1:100	0.1 mL
BSB 3290	Concentrated	1:25 - 1:100	0.5 mL
BSB 3291	Concentrated	1:25 - 1:100	1.0 mL
BSB 3292	Control Slides	Not Applicable	5 slides

Precautions

Presentations

1. For professional users only. Ensure results are interpreted by a medical professional.

2. This product contains sodium azide (NaN3), a toxic chemical which may react with plumbing to form highly explosive build-ups of metal azides. Upon disposal, flush with large volumes of water to prevent sodium azide build-up.

3. Ensure proper handling procedures are used with reagent. Always wear proper laboratory equipment such as laboratory coat and gloves when handling reagents.

4. Unused solution should be disposed of according to local and federal regulations.

5. Do not ingest reagent. If reagent ingested, contact a poison control center immediately.

6. 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" (8).

Storage

Store at 2-8 °**C**. 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.

Specimen Preparation

Paraffin sections: The antibody can be used on formalin-fixed paraffinembedded (FFPE) tissue sections. Ensure tissue undergoes appropriate fixation to ensure 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) or ImmunoDNA Digestor (BSB 0108-0112). See reverse side for complete protocol. Tissue should remain hydrated via use of Bio SB Immuno/DNA Washer solutions (BSB 0029 & BSB 0042).

Frozen sections and cell preparations: The antibody can be used for labeling acetone-fixed frozen sections and acetone-fixed cell preparations.

Staining Procedure

1. Cut and mount 3-5 micron formalin-fixed paraffin-embedded tissues on positive charged slides such as Bio SB Hydrophilic Plus Slides (BSB 7028).

2. Air dry for 2 hours at 58° C.

3. Deparaffinize, dehydrate and rehydrate tissues.

4. Subject tissues to heat epitope retrieval using a suitable retrieval solution such as ImmunoDNA Retriever with Citrate (BSB 0020-BSB 0023) or EDTA (BSB 0030-BSB 0033). 5. 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 in the pressure cooker. Add 1-2 inches of distilled water to the pressure cooker and turn heat to high. Incubate for 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 30-60 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 30-60 minutes.

6. After heat treatment, transfer slides in ImmunoDNA Retriever with Citrate or EDTA to room temperature and let stand for 15-20 minutes.

7. For manual staining, perform antibody incubation at ambient temperature. For automated staining methods, perform antibody incubation according to instrument manufacturer's instructions.

8. Wash slides with IHC wash buffer or DI water.

9. Continue IHC staining protocol.

Recommended IHC Protocol

Step	ImmunoDetector AP/HRP	PolyDetector AP/HRP	PolyDetector Plus HRP	
Peroxidase/AP Blocker	5 min.	5 min.	5 min	
Primary Antibody	30-60 min.	30-60 min.	30-60 min.	
1st Step Detection	10 min.	30-45 min.	15 min.	
2nd Step Detection	10 min.	Not Applicable	15 min.	
Substrate-Chromogen	5-10 min.	5-10 min.	5-10 min.	
Counterstain	Varies	Varies	Varies	

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 medical professional.

References

1. Xia Y, et al. Characterization of the human alpha-synuclein gene: Genomic structure, transcription start site, promoter region and polymorphisms. J. Alzheimers Dis. 2002; 4 (4): 337

2. Uéda K, et al. Molecular cloning of cDNA encoding an unrecognized component of amyloid in Alzheimer disease. Proceedings of the National Academy of Sciences of the United States of America. 1993; 90 (23): 11282–6.

3. Spillantini MG, at al. Alpha-synuclein in Lewy bodies. Nature. 1997; 388 (6645): 839–40.

4. Arima K, at al. Cellular co-localization of phosphorylated tau- and NACP/alpha-synuclein-epitopes in lewy bodies in sporadic Parkinson's disease and in dementia with Lewy bodies. Brain Research. 1999; 843 (1-2): 53–61

5. Arima K, et al. NACP/alpha-synuclein and tau constitute two distinctive subsets of filaments in the same neuronal inclusions in brains from a family of parkinsonism and dementia with Lewy bodies: double-immunolabeling fluorescence and electron microscopic studies. Acta Neuropathologica. 2000; 100 (2): 115–21.

6. Yokota O, et al. NACP/alpha-synuclein, NAC, and beta-amyloid pathology of familial Alzheimer's disease with the E184D presenilin-1 mutation: a clinicopathological study of two autopsy cases. Acta Neuropathologica. 2002;104 (6): 637–48.

7. Singleton AB, et al. alpha-Synuclein locus triplication causes Parkinson's disease. Science. 2003; 302 (5646): 841.

8. 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

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





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