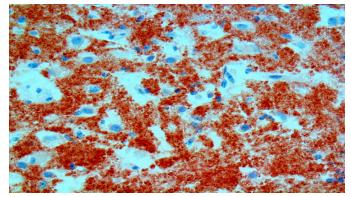


Alpha Synuclein

**Clone:** BSB-114 Mouse Monoclonal







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 formalin-fixed 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 found in the heart, muscles, and other tissues. In the brain, alpha-synuclein 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
Isotype	IgG2a/K	Reactivity	Paraffin, Frozen

Localization	Cytoplasmic, Nuclear	Species Reactivity	Human
Control	Brain, Breast, Skin, Tonsil, Bone Marrow, Alzheimer's Disease, Parkinson's Disease		
Application	Neural & Neuroendocrine Cancer		

#### Presentation

Anti-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 No.	Presentation	Dilution	Volume
BSB 3286	Predilute	Ready-to-Use	3.0 mL
BSB 3287	Predilute	Ready-to-Use	7.0 mL
BSB 3288	Predilute	Ready-to-Use	15.0 mL
BSB 3289	Concentrate	1:25-1:100	0.1 mL
BSB 3290	Concentrate	1:25-1:100	0.5 mL
BSB 3291	Concentrate	1:25-1:100	1.0 mL

#### Control Slides Available

Catalog No.	Quantity
BSB-9011-CS	5 slides

**Storage** Store at 2-8°C (Control Slides: Store at 20-25°C)

#### **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 a laboratory coat, goggles, and gloves when handling reagents.
- 4. Dispose of unused solution with copious amounts 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).

### **Stability**

## This product is stable up to the expiration date on the product label.

Do not use after expiration date listed on the 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 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), 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 on acetone-fixed frozen sections and acetone-fixed cell preparations.

#### **IHC Protocol**

- 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.
- 3. Deparaffinize, dehydrate, and rehydrate tissues.
- 4. 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).
- 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 on trivet 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 IHC, perform antibody incubation at ambient temperature. For automated IHC methods, perform antibody incubation according to instrument manufacturer's instructions.
- 8. Wash slides with ImmunoDNA washer or DI water.
- 9. Continue IHC protocol. Wash slides between each step with ImmunoDNA washer solution.

## **Abbreviated Immunohistochemical Protocol**

Step	ImmunoDetector	PolyDetector	PolyDetector
	AP/HRP	AP/HRP	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 / Coverslip	Varies	Varies	Varies

#### **Mounting Protocols**

For detailed instructions using biodegradable permanent mounting media such as XyGreen PermaMounter (BSB 0169-0174) or organic solvent based resin such as PermaMounter (BSB 0094-0097), refer to PI0174 or PI0097.

#### **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.

#### 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. https://www.cdc.gov/mmwr/pdf/other/su6101.pdf

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