

# Novocastra™ Lyophilized Mouse Monoclonal Antibody Alpha-Synuclein

## Product Code: NCL-ASYN

<b>Intended Use</b>	FOR RESEARCH USE ONLY.
<b>Specificity</b>	Alpha-synuclein, also known as the non-AB component of Alzheimer's disease amyloid precursor protein (NCAP). Does not crossreact with beta-synuclein.
<b>Clone</b>	KM51
<b>Ig Class</b>	IgG1, kappa
<b>Antigen Used for Immunizations</b>	Prokaryotic recombinant protein corresponding to the majority of the full length alpha-synuclein molecule.
<b>Hybridoma Partner</b>	Mouse myeloma (p3-NS1-Ag4-1).
<b>Preparation</b>	Lyophilized tissue culture supernatant containing 15 mM sodium azide. Reconstitute with the volume of sterile distilled water indicated on the vial label.
<b>Effective on Frozen Tissue</b>	Not evaluated.
<b>Effective on Paraffin Wax Embedded Tissue</b>	Yes (using 1 mM EDTA (pH 8.0) unmasking solution combined with the high temperature antigen unmasking technique: see overleaf. Immersion of sections, before or after high temperature antigen unmasking, in 98-100% formic acid for 3 minutes, followed immediately by rinsing in distilled water may enhance staining in some cases).
<b>Recommendations on Use</b>	Immunohistochemistry: Typical working dilution 1:20–1:40. High temperature antigen unmasking technique using 1 mM EDTA (pH 8.0). 60 minutes primary antibody incubation at 25 °C. Standard ABC technique. Western Blotting: Not recommended.
<b>Positive Controls</b>	Immunohistochemistry: Posterior cingulate gyrus (Lewy body dementia).
<b>Staining Pattern</b>	Lewy body and neuropil staining.
<b>Storage and Stability</b>	Store unopened lyophilized antibody at 4 °C. Under these conditions, there is no significant loss in product performance up to the expiry date indicated on the vial label. The reconstituted antibody is stable for at least two months when stored at 4 °C. For long term storage, it is recommended that aliquots of the antibody are frozen at -20 °C (frost-free freezers are not recommended). Repeated freezing and thawing must be avoided. Prepare working dilutions on the day of use.
<b>General Overview</b>	Alpha-synuclein is a protein of 140 amino acids and a member of the synuclein family. It shares sixty one per cent of its sequence with beta-synuclein and is highly conserved between vertebrate species. It does not possess a signal sequence suggesting that it is an intracellular protein. All synucleins have the unusual organisation based around an eleven residue repeating motif and an alpha-helical secondary structure resembling those found in the lipid-binding domain of exchangeable apolipoproteins including Apo E. This homology suggests a direct interaction of alpha-synuclein with membranes consistent with its affinity for synaptosomes. The function of alpha-synuclein may be to carry a target protein to the inner membrane of nerve terminals or to the outer surface of synaptic vesicles.
<b>General References</b>	Baba M, Nakajo S, Tu P-H, et al.. American Journal of Pathology. 152 (4): 879–884 (1998). Polymeropoulos M H, Lavedan C, Leroy E, et al.. Science. 276: 2045–2047 (1997). Spillantini M G, Schmidt M L, Lee V M-Y, et al.. Nature. 388: 839–840 (1997). Iwai A, Masliah E, Sundsmo M P, et al.. Brain Research. 720: 230–234 (1996). Masliah E, Iwai A, Mallory M, et al.. American Journal of Pathology. 148 (1): 201–210 (1996). Weinreb P H, Zhen W, Poon A W, et al.. Biochemistry. 35 (43): 13709–13715 (1996). Xia Y, de Silva H A R, Rosi B L, et al.. Annals of Neurology. 40 (2): 207–215 (1996).



# Instructions for Use

## High Temperature Antigen Unmasking Technique for Immunohistochemical Demonstration on Paraffin Sections

1. Cut and mount sections on slides coated with a suitable tissue adhesive.
2. Deparaffinize sections and rehydrate to distilled water.
3. Place sections in 0.5% hydrogen peroxide/methanol for 10 minutes (or use other appropriate endogenous peroxidase blocking procedure). Wash sections in tap water.
4. Heat 1500 mL of the recommended unmasking solution (0.01 M citrate buffer, pH 6.0 (or Epitope Retrieval Solution, RE7113) unless otherwise indicated overleaf) until boiling in a stainless steel pressure cooker. Cover but do not lock lid.
5. Position slides into metal staining racks (do not place slides close together as uneven staining may occur) and lower into pressure cooker ensuring slides are completely immersed in unmasking solution. Lock lid.
6. When the pressure cooker reaches operating temperature and pressure (after about 5 minutes) start a timer for 1 minute (unless otherwise indicated on the data sheet).
7. When the timer rings, remove pressure cooker from heat source and run under cold water with lid on. DO NOT OPEN LID UNTIL THE INDICATORS SHOW THAT PRESSURE HAS BEEN RELEASED. Open lid, remove slides and place immediately into a bath of tap water.
8. Wash sections in TBS\* buffer (pH 7.6) for 1 x 5 minutes.
9. Place sections in diluted normal serum (or RTU Normal Horse Serum) for 10 minutes.
10. Incubate sections with primary antibody. Use Antibody Diluent RE7133 (where available).
11. Wash in TBS buffer for 2 x 5 minutes.
12. Incubate sections in an appropriate biotinylated secondary antibody.
13. Wash in TBS buffer for 2 x 5 minutes.
14. Incubate slides in ABC reagent (or RTU streptavidin/peroxidase complex).
15. Wash in TBS buffer for 2 x 5 minutes.
16. Incubate slides in DAB or other suitable peroxidase substrate.
17. Wash thoroughly in running tap water.
18. Counterstain with hematoxylin (if required), dehydrate and mount.

### Solutions

0.01 M CITRATE BUFFER (pH 6.0) or RE7113 (where available).

Add 3.84 g of citric acid (anhydrous) to 1.8 L of distilled water. Adjust to pH 6.0 using concentrated NaOH. Make up to 2 L with distilled water.

1 mM EDTA (pH 8.0) or RE7116 (where available).

Add 0.37 g of EDTA (SIGMA product code E-5134) to 1 litre of distilled water. Adjust pH to 8.0 using 1.0 M NaOH.

20 mM TRIS/ 0.65 mM EDTA/ 0.005% TWEEN (pH 9.0) or RE7119 (where available).

Dissolve 14.4 g Tris (BDH product code 271197K) and 1.44 g EDTA (SIGMA product code E-5134) to 0.55 L of distilled water. Adjust pH to 9.0 with 1 M HCl and add 0.3 mL Tween 20 (SIGMA product code P-1379). Make up to 0.6 L with distilled water. This is a 10x concentrate which should be diluted with distilled water as required (eg 150 mL diluted with 1350 mL of distilled water).

\* In most applications, 10 mM phosphate, 0.15 M NaCl, pH 7.6 (PBS) can be used instead of 50 mM Tris, 0.15 M NaCl, pH 7.6 (TBS).

### Safety Note

To ensure the correct and safe use of your pressure cooker, PLEASE READ MANUFACTURER'S INSTRUCTIONS.