Product Datasheet

Human α-Synuclein Fibrils

Lot # 05/23-003.001

Sequence	MDVFMKGLSKAKEGVVAAAEKTKQGVAEAAGKTKEGVL YVGSKTKEGVVHGVATVAEKTKEQVTNVGGAVVTGVTA VAQKTVEGAGSIAAATGFVKKDQLGKNEEGAPQEGILED MPVDPDNEAYEMPSEEGYQDYEPEA
Swiss Prot	P37840
Gene ID	6622
Accession #	NP_000336.1
Species	Human
Amino acids	1-140, full length protein
Conjugates/Tags	No Tag
Molecular weight	14 kDa (14,460 Da)
Nature	Recombinant, expressed in Escherichia coli.
Certificate of analysis	Certified > 95 % purity by SDS-PAGE. Full characterization provided in Figure 1.
Field of Use	Not for use in humans. For research purposes only.
Applications	In vitro assays, cellular assays, animal studies or as standards in WB, SDS-PAGE, ELISA, and other immunoassays.
Form	Shipped in solution on dry ice.
Preparation	Protein was dissolved in PBS at 5 mg/ml.
Storage	Store at -80°C upon receipt. Following resuspension, aliquot and store at -80°C.
Handling	Protein is stable for up to 3 freeze/thaw cycles. We recommend avoiding repeated thawing cycles.
Product Citation	In case of publication or scientific presentations using this product, please cite as Human α-Synuclein Fibrils Polymorph 1 (ND Biosciences SA, Switzerland, Catalogue #ND003, Lot # 05/23-003.001)".
Safety measures	This product is an active protein and may elicit a biological response in vivo, handle with caution.
References	Kumar ST, Donzelli S, Chiki A, Syed MMK, Lashuel HA. A simple, versatile and robust centrifugation-based filtration protocol for the isolation and quantification of α-synuclein monomers, oligomers and fibrils: Towards improving experimental reproducibility in α-synuclein research. J Neurochem. 2020;153(1):103-119. doi:10.1111/jnc.14955.

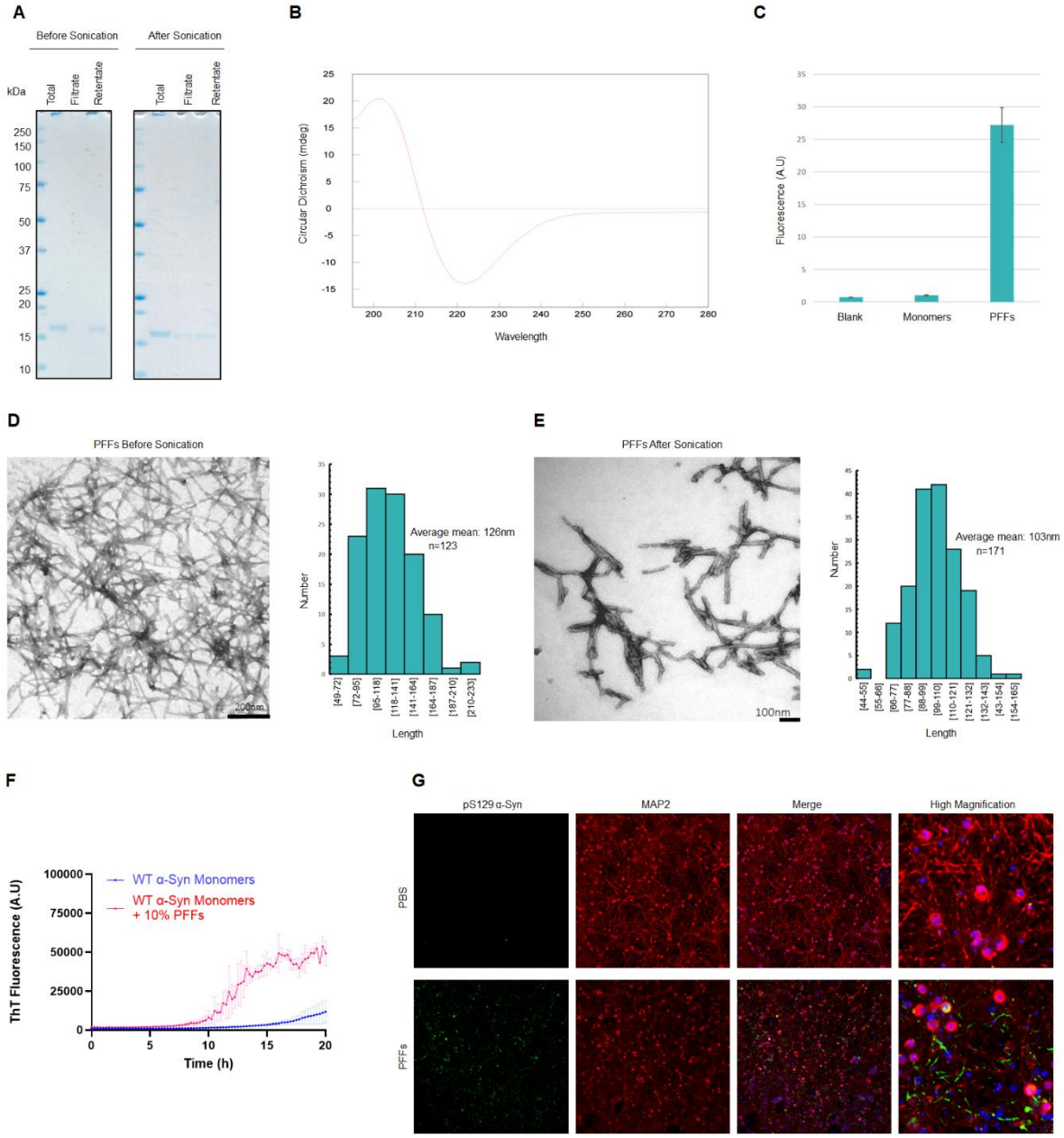

Figure 1. Characterization of α-Syn preformed fibrils (PFFs). (A) Coomassie staining shows that α-Syn PFFs encompass very low amount of released soluble monomers (flow through 100 kDa filter) before and after sonication. Coomassie staining also shows that the α-Syn PFFs migrate as SDS-resistant high molecular weight (HMW) species (in the stacking gel, >250kDa), with some fibrils breaking down into monomers at ~14 kDa. **(B)** Circular dichroism (CD) analysis shows a spectrum with a minimum at 220 nm, establishing β-sheet conformation of α-Syn PFFs after sonication. **(C)** Assessment of ThioflavinT (ThT) binding shows that α-Syn PFFs after sonication binds to ThT and comprise amyloid structure, unlike the blank and α-Syn monomer controls. **(D)** Transmission electron microscopy (TEM) of uranyl acetate stained α-Syn PFFs validates the fibrillar ultrastructure of produced fibrils before sonication and reveals a size distribution between 49 and 233 nm, with an average size of 126 nm. **(E)** TEM analysis validates the fibrillar ultrastructure of sonicated fibrils and reveals a size distribution between 44 and 165 nm, with an average size of 103 nm. **(F)** Assessment of ThT binding (mean ± SD) shows that α-Syn PFFs seed the in vitro aggregation of α-Syn monomers, and eliminate the lag phase. **(G)** Immunocytochemical analysis using the 81A anti-pS129 antibody (Abcam)

shows that α-Syn PFFs are capable of seeding the aggregation of endogenous mouse α-Syn in primary hippocampal neurons 14 days post-treatment. Neurons treated with PBS were analyzed as controls and show no seeding. MAP2 staining was performed to reveal neuronal cell bodies and neurites.

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