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Protocol for α-Synuclein Purification and Ionic Strength Modification Pivotal to High Yield and Reproducibility

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We use this protocol and it's working

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Abstract

Here we describe a protocol for purifying a high yield αSyn protein purification protocol for the efficient production of monomers with a low propensity for self-aggregation. Alpha-synuclein seed amplification assays (αSyn-SAAs) have emerged as promising diagnostic tools for Parkinson's disease (PD) by detecting misfolded αSyn and amplifying the signal through cyclic shaking and resting in vitro. The ultra sensitivity of the assay affords the ability to detect minute quantities of αSyn in peripheral tissues, but it also presents various technical challenges in controlling batch-to-batch variability. To address the problem of variability, we expressed wild type αSyn in BL21 *Escherichia coli*, lysed the cells using osmotic shock, and isolated αSyn using acid precipitation and fast protein liquid chromatography (FPLC). Following purification, we optimized the ionic strength of the reaction buffer to better distinguish the fluorescence maximum (Fmax) separation between disease and healthy control tissues for enhanced assay performance. Our protein purification protocol yielded high quantities of αSyn (average: 68.7 mg/mL per 1 L of culture). Together, these methods are highly reproducible with high purity, stability and yield.

Attachments

DOCX

SYN purification PRO...

2.2MB

Troubleshooting

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