**Soluble and insoluble A-SYN fractionation**

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**ABSTRACT**

Soluble/insoluble alpha-synuclein fractionation is a technique used to separate different forms of the alpha-synuclein protein based on their solubility properties.

**Protocol**

Extraction and detection of Triton-soluble (T-sol) and Triton-insoluble (T-insol) alpha-synuclein was performed as described in Stojkovska and Mazzulli 53. Individual organoids were lysed in 1% Triton X-100 extraction buffer (1% Triton X-100, 150 mM NaCl, 10% glycerol, 25 mM HEPES pH 7.4, 1 mM EDTA, 1.5 mM MgCl2) supplemented with 1X PIC, 50 mM NaF, 2 mM NA3VO4 and 0.5 mM PMSF. Samples were homogenized with a pestle and incubated on a platform shaker in an ice-water slurry for 30 min, followed by three freeze/thaw cycles and ultracentrifugation at 100,000 x g at 4°C for 30 min. The supernatant was removed and labeled T-sol. The remaining pellet was washed in Triton X-100 extraction buffer followed by another ultracentrifugation at 100,000 x g. The pellet was extracted in 2% SDS buffer containing 50 mM Tris, pH 7.4 and 1X PIC, boiled for 10 min at 100°C and labeled the T-insol fraction. T-insol fractions were sonicated in a cup horn probe sonicator (Qsonica – Q700) and boiled again for 10 min at 100°C. The lysate was ultracentrifuged at 100,000 x g for 30 min at 21°C. The supernatant was labeled the SDS-soluble fraction. Protein concentrations were detected using a BCA assay and 30µg of total protein each condition were loaded.