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Soluble and insoluble A-SYN fractionation



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We use this protocol and it's working

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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.

Attachments



[676- 1427.docx](#)

13KB

Materials

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Extraction buffer

	A	B
	Triton X-100	1%
	NaCl	150 mM
	glycerol	10%
	HEPES pH 7.4	25 mM
	EDTA	1 mM
	MgCl ₂	1.5 mM

- 50 mM NaF
- 2 mM Na_3VO_4
- 0.5 mM PMSF
- 50 mM Tris
- cup horn probe sonicator (Qsonica – Q700)



Soluble and insoluble A-SYN fractionation

1h 50m

- 1 Perform extraction and detection of Triton-soluble (T-sol) and Triton-insoluble (T-insol) alpha-synuclein as described in Stojkovska and Mazzulli(2021).
- 2 Lyse individual organoids in 1% Triton X-100 extraction buffer supplemented with 1X PIC,

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- 3 Homogenize samples with a pestle and incubate on a platform shaker in an ice-water slurry for , followed by three freeze/thaw cycles and ultracentrifugation at . 
- 4 Collect the supernatant (Triton-X Soluble fraction).
- 5 Wash the remaining pellet in Triton X-100 extraction buffer followed by another ultracentrifugation at . 
- 6 Resuspend the pellet in 2% SDS buffer containing and 1X PIC, boil it for at (Triton-X insoluble Fraction) and label the T-insol fraction. 



- 7 Sonicate Tx-Insoluble samples for  00:10:00 at 30% power, 20C in a cup horn sonicator (Qsonica-Q700), and then boil them again for  00:10:00 at  100 °C . 
- 8 Ultracentrifuge Tx-Insoluble samples at  100000 x g, 21°C, 00:30:00 . 

- 9 Collect the supernatant (SDS-soluble fraction).
- 10 Detect protein concentrations using a BCA assay and load  30 µg of total protein for each condition.