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 We use this protocol and it's working


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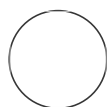
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Soluble and insoluble A-SYN fractionation

 In 1 collection

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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](#)

MATERIALS

Materials

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 NA₃VO₄
- 0.5 mM PMSF
- 50 mM Tris
- cup horn probe sonicator (Qsonica – Q700)

- 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,



[M] 50 millimolar (mM) NaF, [M] 2 millimolar (mM) Na_3VO_4 and [M] 0.5 millimolar (mM) PMSF.

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

- 3 Homogenize samples with a pestle and incubate on a platform shaker in an ice-water slurry for



00:30:00 , followed by three freeze/thaw cycles and ultracentrifugation at










100000 x g, 4°C, 00:30:00 .

1h

- 4 Collect the supernatant (Triton-X Soluble fraction).

- 5 Wash the remaining pellet in Triton X-100 extraction buffer followed by another ultracentrifugation at 100000 x g .



- 6 Resuspend the pellet in 2% SDS buffer containing [M] 50 millimolar (mM) Tris,  7.4 and 1X PIC, boil it for  00:10:00 at  100 °C (Triton-X insoluble Fraction) and label the T-insol fraction. 10m
- 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 . 20m
- 8 Ultracentrifuge Tx-Insoluble samples at  100000 x g, 21°C, 00:30:00 . 30m
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- 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.