



MAR 27, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.8epv5j51511b/v1

Protocol Citation: michela.deleidi, María José Pérez J., Hariam Raji, Pascale Baden, Federico Bertoli 2023. Soluble and insoluble A-SYN fractionation. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.8epv5j51511b/v1>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working
 We use this protocol and it's working

Created: Mar 17, 2023

Last Modified: Mar 27, 2023

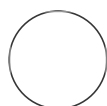
PROTOCOL integer ID:
 78977

Keywords: Soluble A-SYN fractionation, insoluble A-SYN fractionation

🌐 Soluble and insoluble A-SYN fractionation

michela.deleidi¹, María José Pérez J.¹, Hariam Raji¹, Pascale Baden¹, Federico Bertoli¹

¹German Center for Neurodegenerative Diseases (DZNE), Tübingen, 72076 Germany



Federico Bertoli

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 Mazzulli⁵³.


- 2 Lyse individual organoids in 1% Triton X-100 extraction buffer supplemented with 1X PIC,

 50 millimolar (mM) NaF,  2 millimolar (mM) Na_3VO_4 and  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.

- 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
- 
- 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.