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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)

Troubleshooting

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 [53].

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.

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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, pH 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.