Materials List for:

Generation, Purification, and Characterization of Cell-invasive DISC1 Protein Species

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Materials

Name	Company	Catalog Number	Comments
RPMI 1640	Invitrogen	11875-093	Medium is dependent on the host cell line. The optimal recipient cell line should be determined by the user.
DMEM/F-12	Invitrogen	11320-033	Medium is dependent on the recipient cell line. The optimal recipient cell line should be determined by the user.
PBS	Invitrogen	14190-250	
Metafectene	Biontex	T020-1.0	Other transfection reagents might work as well but were not tested with our protocol.
Ni-NTA agarose	Qiagen	30210	The experiments were done with Ni_NTA agarose from Qiagen, other suppliers should work as well.
DNase I	Roche	04716728001	There is no need for RNase free DNase in the process of aggresomes purification.
Sucrose	Sigma-Aldrich	S0389	
Opti-MEM	Invitrogen	31985-062	Serum-free medium works as well
Penicillin/Streptomycin	Invitrogen	15140122	Supplement for SH-SY5Y and NLF medium
Non-essential-amino-acids (NEAA)	Sigma-Aldrich	M7145	Supplement for SH-SY5Y medium
Trypan Blue 0.4%	Sigma-Aldrich	T8154	Toxic reagent
DyLight 594 Maleimide	Thermo-Fisher Scientific	46608	Reduced cysteine reactive dye to form stable thi–ther bonds. Also available in other colors.
ProLong Gold with DAPI	Invitrogen	P36935	This antifade liquid mountant gave superior results in our hands.
Protease Inhibitor cocktail	Roche	11873580001	Dissolve 1 table in 500 µl H_2O for 100X solution.
Synthetic a-synuclein	Sigma	S7820	Refold as described in text.
Table of specific reagents.			
Precellys 24	Bertin Technologies	03119.200.RD000	We have not tested other mechanical homogenizers other than this. Other detergent free homogenization method might work as well, but have not been tested for this protocol.

5301 Concentrator (speedvac)	Eppendorf	5301 000.210	Only necessary if protein has to be concentrated.
LSM 510 and Axiovision Apotome2	Zeiss	See manufacturers catalog	Both microscopes harbor the ability to perform Z-stack imaging. This is a prerequisite for solid and serious evaluation of invasion events.
Cell culture plastic material	Nunc	10 cm dishes #172958, 24-well plates #142475	The use of different plastic material might influence the interaction of recombinant proteins or aggresomes and the plastic surfaces. We have not tested materials other than the ones described here.

Table of specific material and equipment.

Number	Buffer name	content	Comments
1.	Bacterial growth medium	16 g/l Bacto Tryptone, 10 g/l Bacto Yeast extract, 5 g/l NaCl, 5 mM-arginine-HCl, 5mM MGSO4, 100 µg/ml carbencillin, 35 µg/ml chloramphenicol	Grow bacteria to OD_{600}: 0.6-0.8 induce expression with 1 mM IPTG.
2.	Bacterial resuspension buffer	50 mM TRIS-HCl pH 8.0, 5 mM EDTA, 2 mM PMSF, 1% TX-100, 250 µg/ml lysozyme, 20 mM $MgCl_2$ and 400 U/50 ml DNase I	Resupend bacterial pellet from 1 L overnight culture in 50 ml resuspension buffer.
3.	Protein extraction buffer	50 mM Tris pH 8.0, 5 mM imidazole, 500 mM NaCl, 8 M urea and 10 mM b-ME	
4.	Ni-NTA column wash buffer	50 mM Tris pH 8.0, 5 mM imidazole, 500 mM NaCl, 8 M urea and 10 mM b-ME, 12 mM imidazole	Modified extraction buffer with 12 mM imidazole added.
5.	Ni-NTA column elution buffer	50 mM Tris, 500 mM NaCl, 300 mM imidazole, 8 M urea, 1 mM PMSF, 5 mM EDTA and 10 mM b -ME	The elution buffer contains 10 mM b -ME, which is dialyzed later on.
6.	PBS	8 g/l NaCl, 0.2 g/l KCl, 1.44 g/l Na_2HPO_4, 0.24 g/l KH_2PO_4, adjust to pH 7.4	
7.	Labeling buffer	PBS containing 5 mM TCEP	

Table of recipes.