

Materials List for:

Human Peripheral Blood Neutrophil Isolation for Interrogating the Parkinson's Associated LRRK2 Kinase Pathway by Assessing Rab10 Phosphorylation

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Materials

Name	Company	Catalog Number	Comments
1 mL Pipette tips	Sarstedt	70.762	or equivalent
1.5 mL Micro tubes	Sarstedt	72.690.001	or equivalent
10 mL Pipette tips	Sarstedt	86.1254.025	or equivalent
10 µL Pipette tips	Sarstedt	70.113	or equivalent
15 mL falcon tube	Cellstar	188 271	or equivalent
200 µL Pipette tips	Sarstedt	70.760.002	or equivalent
25 mL Pipette tips	Sarstedt	86.1685.001	or equivalent
50 mL falcon tube	Cellstar	227 261	or equivalent
BD Vacutainer Hemogard Closure Plastic K2-EDTA Tube	BD	BD 367525	or equivalent
Beckman Coulter Allegra X-15R centrifuge	Beckman		or equivalent centrifuge with swinging bucket rotator for 15 mL and 50 mL falcon tubes at speed 1000-1200 x g
Category 2 biological safety cabinet.			
cOmplete(EDTA-free) protease inhibitor cocktail	Roche	11836170001	
DIFP (Diisopropylfluorophosphate)	Sigma	D0879	Prepare 0.5M stock solution in isopropanol using special precautions
Dimethyl sulfoxide	Sigma	6250	
Dry ice or liquid nitrogen			
Dulbecco's phosphate-buffered saline	ThermoFisher	14190094	or equivalent
Easy 50 EasySep Magnet	Stemcell	18002	for holding 1 x 50ml conical tube
EasySep Direct Human Neutrophil Isolation Kit	Stemcell	19666	This contains Solutions called "Isolation Cocktail" and "RapidSpheres magnetic beads"
EGTA	Sigma	E3889	
Eppendorf centrifuge 5417R centrifuge	Eppendorf		

Ethanol, in spray bottle			
Ethylenediaminetetraacetic acid	Sigma	E6758	
Ice			
Isopropanol (anhydrous grade)	Sigma	278475	
Lysis buffer (50 mM Tris-HCl pH 7.5, 1%(v/v) Triton X-100, 1 mM EGTA, 1 mM Na ₃ VO ₄ , 50 mM NaF, 10 mM β-glycerophosphate, 5 mM sodium pyrophosphate, 0.27 M sucrose, 0.1% (v/v) β-mercaptoethanol, 1x cOmplete(EDTA-free) protease inhibitor cocktail (Roche), 1 μg/ml Microcystin-LR, 0.5 mM diisopropylfluorophosphate (DIFP).			alternatively frozen lysis buffer in aliquots without Microcystin-LR, DIFP available from MRC-PPU Reagents (http://mrcppureagents.dundee.ac.uk/)
Merck LRRK2 inhibitor II (MLi-2)	Merck	438194-10MG	or equivalent (potent and selective LRRK2 inhinitor)
Microcystin-LR	Enzo Life Sciences	ALX-350-012-M001	1 mg/ml stock in DMSO and store at -80 oC.
Na ₃ VO ₄	Aldrich	450243	
NaF	Sigma	S7920	
Odyssey CLx scan Western Blot imaging system	Odyssey		
Permanent marker pen			
Personal protection equipment			
RPMI 1640 Medium	ThermoFisher	21875034	or equivalent
sodium pyrophosphate	Sigma	S22	
sucrose	Sigma	S0389	
β-glycerophosphate	Sigma	50020	
β-mercaptoethanol	Sigma	M3148	
Suggested antibodies for Western blotting			
Anti-RAB10 (phospho T73) antibody [MJF-R21]	abcam	ab230261	
Anti-α-tubulin	Cell Signaling Technologies	5174	used at 1:2000 dilution
Goat anti-mouse IRDye 680LT	LI-COR	926-68020	used at 1:10,000 dilution
Goat anti-mouse IRDye 800CW	LI-COR	926-32210	used at 1:10,000 dilution
Goat anti-rabbit IRDye 800CW	LI-COR	926-32211	used at 1:10,000 dilution
MJFF-total Rab10 mouse antibody	generated by nanoTools (nanotools.de)	not applicable*	used at 2 μg/ml final concentration; * The MJFF-total Rab10 antibody generated by nanoTools (www.nanotools.de) [11] will be commercialised by the Michael J Fox Foundation in 2018
Mouse anti-LRRK2 C-terminus antibody	Antibodies Incorporated	75-253	used at 1 μg/ml final concentration
pS935-LRRK2	MRC PPU Reagents and Services	UDD2	MJFF-total Rab10 mouse antibody