

Materials List for:

Rab10 Phosphorylation Detection by LRRK2 Activity Using SDS-PAGE with a Phosphate-binding Tag

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Materials

Name	Company	Catalog Number	Comments
Reagents			
Dulbecco's phosphate-buffered saline (DPBS)	homemade		150 mM NaCl, 8 mM Na ₂ HPO ₄ -12H ₂ O, 2.7 mM KCl, 1.5 mM KH ₂ PO ₄ in MilliQ water and sterilized by autoclaving
Sodium chloride	Nacalai Tesque	31320-34	
Sodium Disodium Hydrogenphosphate 12-Water	Wako	196-02835	
Potassium chloride	Wako	163-03545	
Potassium Dihydrogen Phosphate	Wako	169-04245	
2.5% Trypsin (10X)	Sigma-Aldrich	T4549	Dilute 10-fold with sterile DPBS for preparing working solution
Dulbecco's modified Eagle medium (DMEM)	Wako	044-29765	
Fetal bovine serum	BioWest	S1560	Heat-inactivated at 56 °C for 30 min
Penicillin-Streptomycin (100X)	Wako	168-23191	
HEPES	Wako	342-01375	
Sodium hydroxide	Wako	198-13765	
Polyethylenimine HCl MAX, Linear, Mw 40,000 (PEI MAX 40000)	PolySciences, Inc.	24765-1	Stock solution was prepared in 20 mM HEPES-NaOH pH 7.0 at 1 mg/mL and the pH was then adjusted to 7.0 with NaOH
Dimethyl sulfoxide	Wako	045-28335	
Tris	STAR	RSP-THA500G	
Hydrochloric acid	Wako	080-01066	
Polyoxyethylene(10) Octylphenyl Ether	Wako	160-24751	Equivalent to Triton X-100
Ethylene glycol-bis(2-aminoethylether)-N,N,N',N'-tetraacetic acid (EGTA)	Wako	346-01312	
Sodium orthovanadate(V)	Wako	198-09752	
Sodium fluoride	Kanto Chemical	37174-20	
β-Glycerophosphoric Acid Disodium Salt Pentahydrate	Nacalai Tesque	17103-82	
Sodium pyrophosphate decahydrate	Kokusan Chemical	2113899	
Microcystin-LR	Wako	136-12241	

Sucrose	Wako	196-00015	
Complete EDTA-free protease inhibitor cocktail	Roche	11873580001	Dissolve one tablet in 1 mL water, which can be stored at -20 °C for a month. Use it at 1:50 dilution for cell lysis
Pierce Coomassie (Bradford) Protein Assay Kit	Thermo Fisher Scientific	23200	
Sodium dodecyl sulfate	Nacalai Tesque	31607-65	
Glycerol	Wako	075-00616	
Bromophenol blue	Wako	021-02911	
β-mercaptoethanol	Kanto Chemical	25099-00	
Ethanol	Wako	056-06967	
Methanol	Wako	136-01837	
Phosphate-binding tag acrylamide	Wako	AAL-107	P-tag acrylamide
40% (w/v) acrylamide solution	Nacalai Tesque	06119-45	Acrylamide:Bis = 29:1
Tetramethylethylenediamine (TEMED)	Nacalai Tesque	33401-72	
Ammonium persulfate (APS)	Wako	016-08021	10% (w/v) solution was prepared by dissolving the powder of ammonium persulfate in MilliQ water
2-propanol	Wako	166-04831	
Manganese chloride tetrahydrate	Sigma-Aldrich	M3634	
Precision Plus Protein Prestained Standard	Bio-Rad	1610374, 1610373, 1610377	Molecular weight marker used in the protocol
WIDE-VIEW Prestained Protein Size Marker III	Wako	230-02461	
Glycine	Nacalai Tesque	17109-64	
Amersham Protran NC 0.45	GE Healthcare	10600007	Nitrocellulose membrane
Durapore Membrane Filter	EMD Millipore	GVHP00010	PVDF membrane
Filter Papers No.1	Advantec	00013600	
Ponceau S	Nacalai Tesque	28322-72	
Acetic acid	Wako	017-00251	
Tween-20	Sigma-Aldrich	P1379	polyoxyethylenesorbitan monolaurate
Ethylenediaminetetraacetic acid (EDTA)	Wako	345-01865	
Skim milk powder	Difco Laboratories	232100	
Immunostar	Wako	291-55203	ECL solution (Normal sensitivity)
Immunostar LD	Wako	290-69904	ECL solution (High sensitivity)
CBB staining solution	homemade		1 g CBB R-250, 50% (v/v) methanol, 10% (v/v) acetic acid in 1 L of MilliQ water
CBB R-250	Wako	031-17922	
CBB destaining solution	homemade		12% (v/v) methanol, 7% (v/v) acetic acid in 1 L MilliQ water
Name	Company	Catalog Number	Comments
Antibodies			
anti-HA antibody	Sigma-Aldrich	11583816001	Used at 0.2 µg/mL for immunoblotting.
anti-Rab10 antibody	Cell Signaling Technology	#8127	Used at 1:1000 for immunoblotting.

			Specificity was confirmed by CRISPR KO in Ito et al., <i>Biochem J</i> , 2016.
anti-pSer935 antibody	Abcam	ab133450	Used at 1 µg/mL for immunoblotting.
anti-LRRK2 antibody	Abcam	ab133518	Used at 1 µg/mL for immunoblotting.
anti-α-tubulin antibody	Sigma-Aldrich	T9026	Used at 1 µg/mL for immunoblotting.
anti-GAPDH antibody	Santa-Cruz	sc-32233	Used at 0.02 µg/mL for immunoblotting.
Peroxidase AffiniPure Sheep Anti-Mouse IgG (H+L)	Jackson ImmunoResearch	515-035-003	Used at 0.16 µg/mL for immunoblotting.
Peroxidase AffiniPure Goat Anti-Rabbit IgG (H+L)	Jackson ImmunoResearch	111-035-003	Used at 0.16 µg/mL for immunoblotting.
Name	Company	Catalog Number	Comments
Inhibitors			
GSK2578215A	MedChem Express	HY-13237	Stock solution was prepared in DMSO at 10 mM and stored at -80 °C
MLi-2	Provided by Dr Dario Alessi (University of Dundee)		Stock solution was prepared in DMSO at 10 mM and stored at -80 °C
Name	Company	Catalog Number	Comments
Plasmids			
Rab10/pcDNA5 FRT TO HA	Provided by Dr Dario Alessi (University of Dundee)		This plasmid expresses amino-terminally HA-tagged human Rab10.
LRRK2 WT/p3xFLAG-CMV-10	Provided by Dr Takeshi Iwatsubo (University of Tokyo)		Ito et al., <i>Biochemistry</i> , 46: 1380–1388 (2007). This plasmid expresses amino-terminally 3xFLAG-tagged wild-type human LRRK2.
LRRK2 K1906M/p3xFLAG-CMV-10	Provided by Dr Takeshi Iwatsubo (University of Tokyo)		Ito et al., <i>Biochemistry</i> , 46: 1380–1388 (2007). This plasmid expresses amino-terminally 3xFLAG-tagged K1906M kinase-inactive mutant of human LRRK2.
LRRK2 N1437H/p3xFLAG-CMV-10			This paper. This plasmid expresses amino-terminally 3xFLAG-tagged N1437H FPD mutant of human LRRK2.
LRRK2 R1441C/p3xFLAG-CMV-10	Provided by Dr Takeshi Iwatsubo (University of Tokyo)		Kamikawaji et al., <i>Biochemistry</i> , 48: 10963–10975 (2013). This plasmid expresses amino-terminally 3xFLAG-tagged R1441C FPD mutant of human LRRK2.
LRRK2 R1441G/p3xFLAG-CMV-10	Provided by Dr Takeshi Iwatsubo (University of Tokyo)		Kamikawaji et al., <i>Biochemistry</i> , 48: 10963–10975 (2013). This plasmid expresses amino-terminally 3xFLAG-tagged R1441G FPD mutant of human LRRK2.
LRRK2 R1441H/p3xFLAG-CMV-10	Provided by Dr Takeshi Iwatsubo (University of Tokyo)		Kamikawaji et al., <i>Biochemistry</i> , 48: 10963–10975 (2013). This plasmid expresses amino-terminally 3xFLAG-tagged R1441H FPD mutant of human LRRK2.
LRRK2 R1441S/p3xFLAG-CMV-10			This paper. This plasmid expresses amino-terminally 3xFLAG-tagged

			R1441S FPD mutant of human LRRK2.
LRRK2 Y1699C/p3xFLAG-CMV-10	Provided by Dr Takeshi Iwatsubo (University of Tokyo)		Kamikawaji et al., <i>Biochemistry</i> , 48: 10963–10975 (2013). This plasmid expresses amino-terminally 3xFLAG-tagged Y1699C FPD mutant of human LRRK2.
LRRK2 G2019S/p3xFLAG-CMV-10	Provided by Dr Takeshi Iwatsubo (University of Tokyo)		Kamikawaji et al., <i>Biochemistry</i> , 48: 10963–10975 (2013). This plasmid expresses amino-terminally 3xFLAG-tagged G2019S FPD mutant of human LRRK2.
LRRK2 I2020T/p3xFLAG-CMV-10	Provided by Dr Takeshi Iwatsubo (University of Tokyo)		Kamikawaji et al., <i>Biochemistry</i> , 48: 10963–10975 (2013). This plasmid expresses amino-terminally 3xFLAG-tagged I2020T FPD mutant of human LRRK2.
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Equipments			
CO ₂ incubator	Thermo Fisher Scientific	Forma Series II 3110 Water-Jacketed	
Auto Pipette	Drummond	Pipet-Aid PA-400	
Micropipette P10	Nichiryo	00-NPX2-10	0.5–10 µL
Micropipette P200	Nichiryo	00-NPX2-200	20–200 µL
Micropipette P1000	Nichiryo	00-NPX2-1000	100–1000 µL
Tips for micropipette P10	STAR	RST-481LCRST	Sterile
Tips for micropipette P200	FUKAEKASEI	1201-705YS	Sterile
Tips for micropipette P1000	STAR	RST-4810BRST	Sterile
5 mL disporsable pipette	Greiner	606180	Sterile
10 mL disporsable pipette	Greiner	607180	Sterile
25 mL disporsable pipette	Falcon	357535	Sterile
Hematocytometer	Sunlead Glass	A126	Improved Neubauer
Microscope	Olympus	CKX53	
10 cm dishes	Falcon	353003	For tissue culture
6-well plates	AGC Techno Glass	3810-006	For tissue culture
Vortex mixer	Scientific Industries	Vortex-Genie 2	
Cell scrapers	Sumitomo Bakelite	MS-93100	
1.5 mL tubes	STAR	RSV-MTT1.5	
15 mL tubes	AGC Techno Glass	2323-015	
50 mL tubes	AGC Techno Glass	2343-050	
Centrifuges	TOMY	MX-307	
96-well plates	Greiner	655061	Not for tissue culture
Plate reader	Molecular Devices	SpectraMax M2e	
SDS–PAGE tanks	Nihon Eido	NA-1010	
Transfer tanks	Nihon Eido	NA-1510B	
Gel plates (notched)	Nihon Eido	NA-1000-1	
Gel plates (plain)	Nihon Eido	NA-1000-2	
Silicon spacers	Nihon Eido	NA-1000-16	
17-well combs	Nihon Eido	Custom made	
Binder clips	Nihon Eido	NA-1000-15	

5 mL syringe	Terumo	SS-05SZ	
21G	Terumo	NN-2138R	
Power Station 1000 VC	ATTO	AE-8450	Power supply for SDS-PAGE and transfer
Large weighing boats	Ina Optika	AS-DL	
Plastic containers	AS ONE	PS CASE No.4	10 x 80 x 50 mm
Rocking shaker	Titech	NR-10	
Styrene foam box	generic		The internal dimensions should fit one transfer tank (200 x 250 x 250 mm).
ImageQuant LAS-4000	GE Healthcare		An imager equipped with a cooled CCD camera for detection of ECL