

Materials List for

Electroporation-Based Genetic Modification of Primary Human Pigment Epithelial Cells using the Sleeping Beauty Transposon System

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Materials

Name	Company	Catalog Number	Comments
Isolation of primary human RPE cells			
24-Well Cell Culture Plate	Eppendorf, Hamburg, Germany	0030722019	
Amphotericin B [250 µg/mL] (AmphoB)	Merck, Darmstadt, Germany	A2942	
Colibri Forceps	Geuder, Heidelberg, Germany	G-18950	
Curved Iris Forceps	Geuder, Heidelberg, Germany	G-18856	
Disposable Scalpel (No. 11)	Feather, Osaka, Japan		
Dulbecco's Modified Eagle's Medium/Ham's F-12 Nutrient Mixture (DMEM/F12)	PAN-Biotech, Aidenbach, Germany	P04-41150	
Extra Fine Pointed Eye Scissor	Geuder, Heidelberg, Germany	G-19405	
Fetal Bovine Serum [0.2 µm Sterile Filtered] (FBS)	PAN-Biotech, Aidenbach, Germany	P40-37500	
Glass Pasteur Pipettes	Brand, Wertheim, Germany	747715	
Penicillin [10,000 units/mL] and Streptomycin [10 mg/mL] (Pen/Strep)	Merck, Darmstadt, Germany	P0781	
Pipette Tips (1000 µL)	Starlab, Hamburg, Germany		
Single Channel Pipette (100-1000 µL)	Eppendorf, Hamburg, Germany		
Sterile Drape	Lohmann & Rauscher, Rengsdorf, Germany		
Sterile Gauze Compress	Fink-Walter, Merchweiler, Germany	321063	
Sterile Gloves	Sempermed, Wien, Austria		
Sterile Petri Dish (Falcon 60 mm x 15 mm)	Corning, Corning, NY	351007	
Sterile Surgical Gown	Halyard Health, Alpharetta, GA		
Straight Iris Forceps	Geuder, Heidelberg, Germany	G-18855	
Electroporation of primary human RPE cells			
10 mM Tris-HCl (pH 8.5)			

12-Well Cell Culture Plate	Thermo Fisher Scientific, Waltham, MA	150628	
24-Well Cell Culture Plate	Eppendorf, Hamburg, Germany	0030722019	
Amphotericin B [250 µg/mL] (AmphoB)	Merck, Darmstadt, Germany	A2942	
Dulbecco's Modified Eagle's Medium/Ham's F-12 Nutrient Mixture (DMEM/F12)	PAN-Biotech, Aidenbach, Germany	P04-41150	
Safe-Lock Microcentrifuge Tubes (1.5 mL)	Eppendorf, Hamburg, Germany		
Fetal Bovine Serum [0.2 µm Sterile Filtered] (FBS)	PAN-Biotech, Aidenbach, Germany	P40-37500	
Inverted Microscope	Leica Mikrosysteme, Wetzlar, Germany	Leica DMI8	
Microvolume Spectrophotometer (NanoDrop Spectrophotometer)	Thermo Fisher Scientific, Waltham, MA		
Capillary Transfection System (Neon Transfection System)	Thermo Fisher Scientific, Waltham, MA	MPK5000	
Neon Transfection System 10 µL Kit	Thermo Fisher Scientific, Waltham, MA	MPK1096	
Hemocytometer (Neubauer Chamber)	Paul Marienfeld, Lauda-Königshofen, Germany	0640110	
PBS Dulbecco w/o Ca ²⁺ w/o Mg ²⁺	Biochrom, Berlin, Germany	L182-50	
Penicillin [10,000 units/mL] and Streptomycin [10 mg/mL] (Pen/Strep)	Merck, Darmstadt, Germany	P0781	
Pipette Tips (10 µL)	Starlab, Hamburg, Germany		
Pipette Tips (1000 µL)	Starlab, Hamburg, Germany		
Pipette Tips (200 µL)	Starlab, Hamburg, Germany		
Plasmid Maxi Kit	Qiagen, Hilden, Germany	12163	
Single Channel Pipette (0.1-10 µL)	Eppendorf, Hamburg, Germany		
Single Channel Pipette (100-1000 µL)	Eppendorf, Hamburg, Germany		
Single Channel Pipette (10-200 µL)	Eppendorf, Hamburg, Germany		
Trypan Blue Solution	Merck, Darmstadt, Germany	T8154	
Trypsin-EDTA (0,05 %)	Thermo Fisher Scientific, Waltham, MA	25300054	
Analyses of transfected primary human RPE cells			
10% SDS-Polyacrylamide Gel			
1x Incubation Buffer (50 mM NaH ₂ PO ₄ , 300 mM NaCl, 10 mM imidazole, pH 8.0)			
2x SDS Sample Buffer			
4x Incubation Buffer (200 mM NaH ₂ PO ₄ , 1.2 M NaCl, 40 mM imidazole, pH 8.0)			
Amersham Protran Supported 0.2 µm Nitrocellulose Blotting Membrane	Cytiva, Marlborough, MA	10600015	
Amphotericin B [250 µg/mL] (AmphoB)	Merck, Darmstadt, Germany	A2942	

Anti-PEDF Antibodies (Rabbit Polyclonal)	BioProducts, Middletown, MD	AB-PEDF1	
Anti-Penta-His Antibodies (Mouse Monoclonal)	Qiagen, Hilden, Germany	34660	
Dulbecco's Modified Eagle's Medium/Ham's F-12 Nutrient Mixture (DMEM/F12)	PAN-Biotech, Aidenbach, Germany	P04-41150	
Elution Buffer (50 mM NaH ₂ PO ₄ , 300 mM NaCl, 250 mM imidazole, pH 8.0)			
Fetal Bovine Serum [0.2 µm Sterile Filtered] (FBS)	PAN-Biotech, Aidenbach, Germany	P40-37500	
Hemocytometer (Neubauer Chamber)	Paul Marienfeld, Lauda-Königshofen, Germany	0640110	
Horseradish Peroxidase-Conjugated Anti-Mouse Antibodies (Rabbit Polyclonal)	Agilent Dako, Santa Clara, CA	P0260	
Horseradish Peroxidase-Conjugated Anti-Rabbit Antibodies (Goat Polyclonal)	Abcam, Cambridge, United Kingdom	ab6721	
Human PEDF ELISA Kit	BioProducts, Middletown, MD	PED613	
LAS-3000 Imaging System	Fujifilm, Minato, Japan		
LightCycler 1.2 Instrument	Roche Life Science, Penzberg, Germany		
LightCycler FastStart DNA Master SYBR Green I	Roche Life Science, Penzberg, Germany	12239264001	
LightCycler Capillaries (20 µl)	Roche Life Science, Penzberg, Germany	4929292001	
Microvolume Spectrophotometer (NanoDrop Spectrophotometer)	Thermo Fisher Scientific, Waltham, MA		
Mini-PROTEAN Tetra Cell Casting Module	Bio-Rad Laboratories, Feldkirchen, Germany	1658015	
Mini-PROTEAN Tetra Vertical Electrophoresis Cell for Mini Precast Gels, 4-gel	Bio-Rad Laboratories, Feldkirchen, Germany	1658004	
Ni-NTA Superflow	Qiagen, Hilden, Germany	30410	
PageRuler Prestained Protein Ladder	Thermo Fisher Scientific, Waltham, MA	26616	
Penicillin [10,000 units/mL] and Streptomycin [10 mg/mL] (Pen/Strep)	Merck, Darmstadt, Germany	P0781	
Pipette Tips (10 µL)	Starlab, Hamburg, Germany		
Pipette Tips (1000 µL)	Starlab, Hamburg, Germany		
Pipette Tips (200 µL)	Starlab, Hamburg, Germany		
PowerPac Basic Power Supply	Bio-Rad Laboratories, Feldkirchen, Germany	1645050	
QIAamp DNA Mini Kit	Qiagen, Hilden, Germany	51304	
Reverse Transcription System	Promega, Madison, WI	A3500	
RNase-Free DNase Set	Qiagen, Hilden, Germany	79254	
RNeasy Mini Kit	Qiagen, Hilden, Germany	74104	
Rocking Shaker	Cole-Parmer, Staffordshire, United Kingdom	SSM3	
Safe-Lock Microcentrifuge Tubes (1.5 mL)	Eppendorf, Hamburg, Germany		

Safe-Lock Microcentrifuge Tubes (2.0 mL)	Eppendorf, Hamburg, Germany		
Single Channel Pipette (0.1-10 µL)	Eppendorf, Hamburg, Germany		
Single Channel Pipette (100-1000 µL)	Eppendorf, Hamburg, Germany		
Single Channel Pipette (10-200 µL)	Eppendorf, Hamburg, Germany		
Trans-Blot Turbo Transfer System	Bio-Rad Laboratories, Feldkirchen, Germany	1704150	
Trypan Blue Solution	Merck, Darmstadt, Germany	T8154	
Trypsin-EDTA (0,05 %)	Thermo Fisher Scientific, Waltham, MA	25300054	