

Materials List for:

An Ex Vivo Tissue Culture Model for Fibrovascular Complications in Proliferative Diabetic Retinopathy

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Materials

Name	Company	Catalog Number	Comments
Material			
Microforceps	Medicon	07.60.03	Used for handling the FTs
Disposable Scalpels - Sterile	Swann-Morton	0513	Used for FT dissection
Culture dish, vented, 28 ml (60mm)	Greiner Bio-One	391-3210	Used for dissection and for testing fibrin gel formation
Cell culture plates, 12-well	Greiner Bio-One	392-0049	Used for FT dissection and whole-mount immunofluorescence
Reagent/centrifuge tube with screw cap, 15 mL	Greiner Bio-One	391-3477	
Reagent/centrifuge tube with screw cap, 50 mL	Greiner Bio-One	525-0384	
Millex-GV Syringe Filter Unit, 0.22 µm, PVDF	Millipore	SLGV033RS	Used to sterile-filter the fibrinogen solution
Syringe, 10 mL	Braun	4606108V	Used to sterile-filter the fibrinogen solution
Polypropylene Microcentrifuge Tubes, 1.5 mL	Fisher	FB74031	
Cell-Culture Treated Multidishes, 24-well	Nunc	142475	Used for casting the FT/fibrin gels for native FT characterization and ex vivo culture
Cell culture plates, 96-well, U-bottom	Greiner Bio-One	392-0019	Used for whole-mount immunofluorescence
Round/Flat Spatulas, Stainless Steel	VWR	82027-528	Used for whole-mount immunofluorescence
Coverslips 22x22mm #1	Menzel/Fisher	15727582	Used for mounting
Microscope slides	Fisher	Kindler K102	Used for mounting
Absorbent paper	VWR	115-0202	Used for mounting
Name	Company	Catalog Number	Comments
Reagents			
PBS tablets	Medicago	09-9400-100	Used for preparing 1x PBS
Fibrinogen, Plasminogen-Depleted, Human Plasma	Calbiochem	341578	
Hanks Balanced Salt Solution	Sigma-Aldrich	H9394-500ML	Used for preparing the fibrinogen and TA solution
Fetal bovine serum	Gibco	10270106	Used for preparing the blocking solution

Human Serum	Sigma-Aldrich	H4522	Aliquoted in -20 °C, thaw before preparing the <i>ex vivo</i> culture media
Gentamicin Sulfate 10mg/ml	Biowest	L0011-100	
Endothelial cell media MV Kit	Promocell	C-22120	Contains 500 ml of Endothelial Cell Growth Medium MV, 25 mL of fetal calf serum, 2 mL of endothelial cell growth supplement, 500 µL of recombinant human epidermal growth factor (10 µg/ mL) and 500 µL of hydrocortisone (1 g/ mL)
Sodium azide	Sigma-Aldrich	S2002	Used for storage of the native and <i>ex vivo</i> cultured FTs. TOXIC: wear protective gloves and/or clothing, and eye and/or face protection. Use in fume hood.
Acetone	Sigma-Aldrich	32201-2.5L-M	Used to prepare the post-fixation solution. HARMFUL: wear protective gloves and/or clothing. Use in fume hood.
Methanol	Sigma-Aldrich	32213	Used to prepare the post-fixation solution. TOXIC: wear protective gloves and/or clothing. Use in fume hood.
Triton X-100 (octyl phenol ethoxylate)	Sigma-Aldrich	T9284	Used for whole-mount immunofluorescence. HARMFUL: wear protective gloves and/or clothing.
Hoechst 33342, 20mM	Life Technologies	62249	For nuclei counterstaining. HARMFUL: wear protective gloves and/or clothing, and eye and/or face protection.
VECTASHIELD Antifade Mounting Medium	Vector Laboratories	H-1000	Wear protective gloves and/or clothing, and eye protection. Use in fume hood.
VECTASHIELD Antifade Mounting Medium with DAPI	Vector Laboratories	H-1200	Mounting medium with nuclei counterstaining. Wear protective gloves and/or clothing, and eye protection. Use in fume hood.
Eukitt Quick-hardening mounting medium	Sigma-Aldrich	03989-100ml	TOXIC: Wear protective gloves and/or clothing, and eye protection. Use in fume hood.
Thrombin from bovine plasma, lyophilized powder	Sigma-Aldrich	T9549-500UN	Dissolve at 100 units/ mL, aliquote and store at -20 °C, avoid repeated freeze/ thaw
Aprotinin from bovine lung, lyophilized powder	Sigma	A3428	Dissolve at 50 mg/ mL, aliquote and store at -20 °C, avoid repeated freeze/ thaw
Name	Company	Catalog Number	Comments
Growth factors			
Recombinant human VEGFA	R&D Systems	293-VE-010	50 ng/ mL final concentration
Recombinant human VEGFC	R&D Systems	752-VC-025	200 ng/ mL final concentration
Recombinant human TGFβ	Millipore	GF346	1 ng/ mL final concentration
Recombinant human bFGF	Millipore	01-106	50 ng/ mL final concentration
Name	Company	Catalog Number	Comments
Primary antibodies			
CD31 (JC70A)	Dako	M0823	Used at 1:100 dilution, Donkey anti Mouse Alexa 488 Secondary Ab

CD34 (QBEND10)	Dako	M716501-2	Used at 1:100 dilution, Donkey anti Mouse Alexa 488 Secondary Ab
CD45 (2B11+PD7/26)	Dako	M070129-2	Used at 1:100 dilution, Donkey anti Mouse Alexa 488 Secondary Ab
CD68	ImmunoWay	RLM3161	Used at 1:100 dilution, Donkey anti Mouse Alexa 488 Secondary Ab
Cleaved caspase-3 (5A1E)	Cell Signalling	9664	Used at 1:200 dilution, Goat anti Rabbit Alexa 594 Secondary Ab
ERG (EP111)	Dako	M731429-2	Used at 1:100 dilution, Goat anti Rabbit Alexa 594 Secondary Ab
GFAP	Dako	Z0334	Used at 1:100 dilution, Goat anti Rabbit Alexa 594 Secondary Ab
Ki67	Leica Microsystems	NCL-Ki67p	Used at 1:1500 dilution, Goat anti Rabbit Alexa 594 Secondary Ab
Lyve1	R&D Systems	AF2089	Used at 1:100 dilution, Donkey anti Goat Alexa 568 Secondary Ab
NG2	Millipore	AB5320	Used at 1:100 dilution, Goat anti Rabbit Alexa 594 Secondary Ab
Prox1	ReliaTech	102-PA32	Used at 1:200 dilution, Goat anti Rabbit Alexa 568 Secondary Ab
Prox1	R&D Systems	AF2727	Used at 1:40 dilution, Chicken anti Goat Alexa 594 Secondary Ab
VEGFR3 (9D9F9)	Millipore	MAB3757	Used at 1:100 dilution, Donkey anti Mouse Alexa 488 Secondary Ab
α -SMA (1A4)	Sigma	C6198	Used at 1:400 dilution, Cy3 conjugated
Name	Company	Catalog Number	Comments
Secondary antibodies			
Alexa Fluor488 Donkey Anti-Mouse IgG	Life Technologies	A-21202	Used at 1:500 dilution
Alexa Fluor594 Goat Anti-Rabbit IgG	Invitrogen	A-11012	Used at 1:500 dilution
Alexa Fluor568 Donkey anti-Goat IgG	Thermo Scientific	A-11057	Used at 1:500 dilution
Alexa Fluor568 Goat anti-Rabbit IgG	Thermo Scientific	A-11036	Used at 1:500 dilution
Alexa Fluor594 Chicken Anti-Goat IgG	Molecular Probes	A-21468	Used at 1:500 dilution
Name	Company	Catalog Number	Comments
Microscopes			
Axiovert 200 inverted epifluorescence microscope	Zeiss		For imaging of the fresh and fibrin-embedded FT
SZX9 upright dissection stereomicroscope	Olympus		For FT dissection
LSM 780 confocal microscope	Zeiss		For imaging of whole-mount immunostained FT
Axiomager.Z1 upright epifluorescence microscope with Apotome	Zeiss		For imaging of whole-mount immunostained FT