

Materials List for:

Ex Vivo Corneal Organ Culture Model for Wound Healing Studies

Nileyma Castro^{*1}, Stephanie R. Gillespie^{*2}, Audrey M Bernstein¹

¹Department of Ophthalmology, SUNY Upstate Medical University

²Department of Dermatology, Columbia University Medical Center

*These authors contributed equally

Correspondence to: Audrey M Bernstein at bernstea@upstate.edu

URL: <https://www.jove.com/video/58562>

DOI: [doi:10.3791/58562](https://doi.org/10.3791/58562)

Materials

Name	Company	Catalog Number	Comments
PBS	Gibco	10-010-023	
Pen Strep	MP Biomedicals	91670049	
Bovine Collagen Solution	Advance Biomatrix	5005	
Pig eyes with lids attached	Pel-freeze, Arkansas	N/A	
6.0 mm trephine	Katena	K28014	
Surgical Blade	Personna	0.009	
Small scissor	Fisher	895110	
Forceps	Fisher	08953-F	
Kim Wipes	Kimberly-Clark™ 34120	06-666	
60 mm cell culture dishes	Falcon	08-772B	
Supplemented Serum- Free media (SSFM)			Add all of the following components to DMEM/F-12: ITS, RPMI, Glutathione, L-Glutamine, MEM Non essential amino acids, MEM Sodium Pyruvate, ABAM, Gentamicin, Vitamin C.
DMEM/F-12	Gibco	11330	
ITS Liquid Media Supplement	Sigma	I3146	100X
RPMI 1640 Vitamins Solution	Sigma	R7256	100X
Glutathione	Sigma	G6013	Use at 1 µg/mL. Freeze aliquots; do not reuse after thawing.
1% L-glutamine solution	Gibco	25030-081	100X
MEM Non-essential amino acids solution	Gibco	11140	100X
MEM Sodium pyruvate solution	Gibco	11360	1 M Stocks (1000X) and freeze in single use aliquots. Use from freezer each time media is made.
ABAM	Sigma	A7292	100X
Gentamicin	Sigma	30-005-CR	200X
Vitamin C	Wako	070-0483	2-O-aD Glucopyranosyl-Ascorbic Acid. 1 mM stocks (1000x)
10% Iodine	Fisher Chemical	SI86-1	
Tissue Path Cassettes	Fisher	22-272416	
Normal Goat Serum (NGS)	Jackson Immuno Research	005-000-121	We use 3% NGS
Mounting Media	Thermo Scientific	TA-030-FM	
Safe Clear	Fisher	314-629	

Ethyl Alcohol	Ultra Pure	200CSGP	200 Proof, diluted at 100%, 70%, 50%)
Sodium citrate	Fisher	BP327	10mM, pH 6.4
Hematoxylin	EMD Millipore	M10742500	
Bluing agent	Ricca Chemical Company	220-106	
1% Triton X-100	Fisher	9002-93-1	Diluted in PBS
0.1% Tween 20	Fisher	BP337	Diluted in PBS
3% Hydrogen Peroxide	Fisher	H324	
DAB Kit	Vector Laboratories	SK-4100	
Agar	Fisher	BP1423-500	Agar solution: prepare 1% agar and 1 mg/mL bovine collagen in DMEM-F12 up to 20 mL
Parafilm	Bermis	13-374-12	
Moist Chamber			Use any chamber, cover it with wet Wipe Tissue and then put a layer of Parafilm over it.
Lipofectamine 2000			
Qiagen RNeasy Protect Cell Reagent	Qiagen	76104	
Ambion PureLink RNA Mini Kit	Thermo Scientific	12183018A	
Anti-Fibronectin-EDA Antibody	Sigma	F6140	1:200 Diluted in 3% normal goat serum
Anti-alpha smooth muscle actin Antibody	Sigma	A2547 or C6198 (cy3 conjugated)	1:200 Diluted in 3% normal goat serum
Permafluor	Thermo Scientific	TA-030-FM	
DAPI	Invitrogen	P36931	
Gt anti -MS IgG (H+L) Secondary Antibody, HRP	Invitrogen	62-6520	1:100 diluted in 3% normal goat serum (for a-SMA, DAB staining)
Gt anti -MS IgM (H+L) Secondary Antibody, HRP	Thermo Scientific	PA1-85999	1:100 diluted in 3% normal goat serum (for FN-EDA, DAB staining)
Gt anti -MS IgG (H+L) Secondary Antibody, Cy3	Jackson Immuno Research	115-165-146	1:200 Diluted in 3% normal goat serum (for a-SMA, Fluorescence staining)
Zeiss Axioplan2	Zeiss		Microscope
SPOT-2	Diagnostic Instruments, Sterling Heights, Michigan	CCD camera	